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CONTENTS

SCIENTIFIC PROCEEDINGS, VOLUME 39:

Page

Two hundred and sixty-fifth issue, October, 1938, No. 1 1

Two hundred and sixty-sixth issue, November, 1938, No. 2 227

Two hundred and sixty-seventh issue, December, 1938, No. 3 415

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NEW YORK	
New York Academy of Medicine	October 26, 1938
PEIPING	
Peiping Union Medical College	June 1, 1938
SOUTHERN CALIFORNIA AND PACIFIC COAST	
San Diego, Calif.	June 22, 1938

10068 P

Effect of Roentgen Rays on the Phytopharmacological Reaction of Blood.

David I. Macht.

From the Pharmacological Research Laboratory, Hynson, Westcott & Dunning, Inc., Baltimore, Md.

Roentgen sickness is a not infrequent occurrence among patients undergoing X-ray therapy. The profoundly toxic reaction of an English setter irradiated for the purpose of sterilization was the starting-point of the present investigation. The question raised was whether a toxic substance, possibly generated by such irradiation, could be detected in the blood of various animals. Inasmuch as toxins which could not be demonstrated by zoöpharmacological methods have been detected by the phytopharmacological test,[1, 2] such a phytopharmacological examination was made of blood specimens obtained before and after exposure of various animals to

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[1] Macht, D. I., and Livingston, M. B., *J. Gen. Physiol.*, 1922, **4**, 573.

[2] Macht, D. I., *Science*, 1930, **71**, 302.

X-rays. The method employed has been described in numerous publications.[3, 4] The effect of one percent solutions of whole blood or of blood serum on roots of *Lupinus albus* seedlings, grown under standard conditions of temperature, light, etc., was observed and recorded.

Blood from 10 kinds of animals was studied and this preliminary announcement reports the results obtained in experiments with rats (30), rabbits (25), cats (5), dogs (2), guinea pigs (4), mice (10), pigeons (3), fish (2), frogs (6), and human beings (2). The figures in parentheses indicate the number of experiments performed. The effect of one percent solutions of either whole blood of smaller animals or blood serum of larger animals in normal condition was compared with that of one percent solutions of blood or serum obtained after exposure of the same animals to various doses of X-rays. The physical apparatus used in this investigation through the courtesy and collaboration of Dr. C. F. Burnam of the Howard A. Kelly Hospital was a constant potential electric transformer with water-cooled therapy tubes. The kilovoltage was 264, milliamperes, 30. The X-rays were filtered through a copper plate and an aluminum plate, each one millimeter thick. Animals were irradiated at a distance of 50 cm, dosage per minute being 105r. The dosage used ranged from 200r to 750r and occasionally even to 1000r. In some experiments the effect of small *repeated* doses of X-rays was also studied.

Table I sets forth the clear-cut and striking results obtained. When normal specimens from the various animals were compared phytopharmacologically with those taken after their irradiation, it was found that Roentgen rays rendered the blood more toxic for *Lupinus albus* seedlings. The degree of toxicity varied considerably, depending on the dosage of radiation used. More interesting, however, was the fact that this toxic substance did not usually appear in the blood immediately after irradiation. Some toxicity was detected in blood drawn a few hours after exposure but the greatest amount was usually noted 24 hours after exposure. This finding suggests the gradual formation and liberation of a toxic substance as a result of irradiation of various tissues. The toxemia persisted for 2 or 3 days after which the animals' blood returned to normal unless the X-ray dosage had been very great. Most of the tests were made on lower animals in normal health. Results of clinical studies of patients undergoing X-ray therapy, which are now in progress, will

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[3] Macht, D. I., *Protoplasma*, 1936, **27**, 1.

[4] Macht, D. I., *Arch. Derm. and Syphilol.*, 1937, **36**, 1022.

be reported later. The blood of the patient listed in the table, Mrs. Y, with advanced carcinoma of the breast, showed over 50% toxicity the day following her exposure to 300r for therapeutic purposes. In such clinical cases, of course, the phytotoxic reaction may be due in large measure to the products of decomposition elaborated by the malignant growth as the result of irradiation.

TABLE I.

Animal	Dosage r	Normal Phytotoxic Index*	%	Phytotoxic Index* After Irradiation	%
Mouse	250	Whole blood	74	After 24 hr	52
Rat	300	,, ,,	77	,, ,, ,,	52
Guinea pig	250	Blood serum	76	,, ,, ,,	56
Kitten	250	,, ,,	71	,, ,, ,,	54
Rabbit	300	,, ,,	70	,, one ,,	68
,,	300	,, ,,	70	,, 24 ,,	55
,,	300	,, ,,	70	,, 72 ,,	48
,,	300	,, ,,	70	,, one week	75
Pigeon	250	Whole blood	63	,, 24 hr	47
Dog	300	Blood serum	75	,, ,, ,,	66
Fish (pike)	500	,, ,,	75	,, ,, ,,	64
Frog	500	Whole blood	55	,, 2½ ,,	27
,,	500	,, ,,	55	,, 24 ,,	23
Mrs. Y. (Carcinoma of breast)	300	Blood serum	68	,, ,, ,,	32

* Ratio of growth in blood solution to growth in physiological control solution.

Studies are being made on the relative toxicity of blood obtained after irradiation of different regions of the animal body. The possible value of some substances suggested as antidotes for Roentgen sickness as, for instance, Congo red, various vitamins and extracts from *Melanoplus differentialis,* a grasshopper rich in several vitamins,[5] is also being investigated.

Summary. Phytopharmacological experiments on samples of blood obtained from normal animals before and after their exposure, respectively, to various doses of Roentgen rays revealed that they contain a substance which inhibits root growth. This substance may be detected a few hours after irradiation but is found in largest amounts 24 hours later. The effect of irradiation of different parts of the animal body on the toxicity of the blood, the possibly antidotal effect of some chemical compounds and the clinical aspects of the subject in human beings are still under investigation.

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[5] Macht, D. I., *Am. J. Physiol.,* 1937, **119**, 268.

10069 P

Effect of Synthetic Male Hormone Substance on Descent of Testicles in Human Cryptorchidism.*

James B. Hamilton and Gilbert Hubert. (Introduced by Edgar Allen.)

From the Department of Anatomy, Yale University School of Medicine, and the Department of Pediatrics, Albany Hospital.

Anterior pituitary-like substance has been employed with some degree of success in a certain percentage of cases of cryptorchidism.[1] The action is possibly effected through stimulation of the production of male hormone,[2] since male hormone (androgen) is responsible at least in part for testicular descent in monkeys,[3] rats,[4] and as shown in the present report, humans.

Thirty-two cryptorchid males between the ages of 1½ and 27 years received subcutaneous injections of 5 to 20 mg of testosterone propionate† 3 to 7 times weekly, the amount being adjusted to the size and age of the individual and the response to the material. Standard tests[5] were used to differentiate spastic from true retention of the testicle, a procedure vital for the evaluation of the results of any therapy. Twelve cases were pseudocryptorchid (spastic retention); 20 presented a state of true cryptorchidism, 8 bilaterally, 12 unilaterally. There was indication of endocrine abnormality in 8, of familial endocrine disturbance in 6.

Eight cases of bilateral and 9 of unilateral true cryptorchidism have received what is believed to be an adequate course of treatment, the maximum length of which was 5 months.

Descent of the testes with satisfactory placement in the bottom of the scrotum occurred in 1 out of 9 unilateral cases of cryptorchidism, and as follows in the bilateral cases: complete bilateral descent in 3, unilateral descent in 2, partial descent in 2; the remaining case was a hypogonad whose testes were not identified prior to treat-

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* Aided by grants from the Committee on Scientific Research of the American Medical Association and the International Cancer Research Foundation.

[1] Shapiro, B., *Z. f. klin. Med.*, 1930, **114**, 610; Engle, E. T., *Endocrinology*, 1932, **16**, 513.

[2] Hamilton, J. B., *Am. J. Physiol.*, 1937, **119**, 325.

[3] Hamilton, J. B., *Anat. Rec.*, 1938, **70**, 533.

[4] Hamilton, J. B., and Leonard, S. L., *Anat. Rec.*, 1938, **71**, 105.

† Testosterone propionate was furnished through the courtesy of the Ciba Company under the trade-name Perandren.

[5] Hamilton, J. B., and Hubert, G., *Endocrinology*, 1935, **21**, 644.

ment, but after injections were located in the scrotum.[6] Surgical means employed in 5 unilateral and 2 bilateral cases not responding to hormone treatment, showed adhesions or other mechanical factors preventing descent in all operated subjects. Penile erections, increased vascularity of the nasal mucosa, and growth of the scrotum, prostate and genital hair were observed. There was no lowering of vocal pitch. Twelve cases of pseudocryptorchidism treated with amounts of male hormone too small to give penile and scrotal growth were sufficient to condition the testes against spastic retention.

10070

Relation Between Size of Dose and Lipotropic Effect of Choline Chloride in Mice.*

Mary Scott Welch and Arnold DeM. Welch. (Introduced by C. F. Cori.)

From the Department of Pharmacology, Washington University School of Medicine, St. Louis.

In recent years Best and his coworkers have established the effectiveness of choline chloride in preventing (or curing) fatty infiltration of the liver in depancreatized dogs and in rats on a low choline-high fat diet. Preliminary results with mice, chickens and puppies were reported by Best, Huntsman and Solandt,[1] but the choice of animals for choline studies has been restricted essentially to the rat and depancreatized dog.

We have found the mouse an excellent species for the study of the lipotropic action of choline chloride and related compounds, in many ways preferable to the rat. It is possible to complete an experiment satisfactorily in 5 or 6 days, while it has been the custom to conduct rat experiments for 2 to 4 weeks before sacrificing the animals. The reduction in the amount of low choline diet required and of difficultly prepared or expensive compounds related to choline chloride is important also.

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[6] Hamilton, J. B., *Endocrinology,* 1937, **21**, 649.

* This investigation has been made with the assistance of a grant from the Committee on Therapeutic Research, Council on Pharmacy and Chemistry, American Medical Association. The authors wish to acknowledge their appreciation of the interest and suggestions of Prof. C. F. Cori.

[1] Best, C. H., Huntsman, M. E., and Solandt, O. M., *Trans. Roy. Soc. Canada,* 1932, Section V, 175.

Male mice of the same strain and of approximately the same age and weight (15-25 g) are arranged in groups of 8-12 in the experiments described and kept on a standard diet until the day previous to the beginning of the experiment. During the experimental period the diet consists of powdered beef muscle (Difco), well extracted with alcohol and ether, 5.3%; powdered sucrose, 48%; beef fat, 38%; cod liver oil, 2%; agar, 2%; salt mixture (U.S.P. XI No. 2), 4%; yeast concentrate,† 0.75%. The chemical to be tested is dissolved in a small amount of water and very thoroughly incorporated with the food; fresh supplies of the latter are placed daily in beakers (50 cc) which are wired to the cages; the weight of food residues is recorded daily. The animals waste little of their food supply when fed 2 g per mouse per day. The change in weight during a 6-day period may vary between ±10% but usually shows a small gain. The animals are decapitated and the livers analyzed for "total fat," which consists of the weight of the residue from the petroleum ether soluble fraction of the pooled livers, following saponification and acidification.

The efficacy of choline chloride in preventing deposition of "fat" in the livers of the mice may be expressed as follows: $\frac{x-y}{x-3.5} \cdot 100 =$ percentage effectiveness of test dose; where x represents the percentage of "fat" in the livers of control animals; y, the percentage of "fat" in the livers of animals which received choline chloride and 3.5 the average percentage of "fat" in the livers of normal mice.

In Fig. 1 A the results of such calculations are plotted against

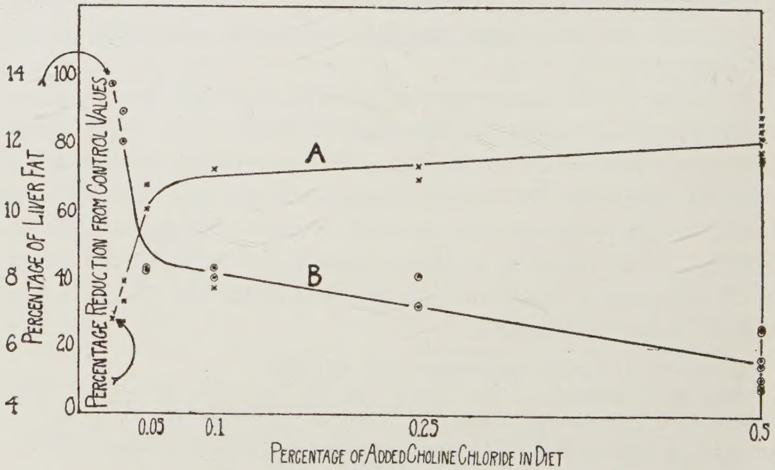

FIG. 1.

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† Generously supplied by Anheuser-Busch, Inc.

dosage of choline chloride. In Fig. 1 B the actual percentage of liver fat in the test animals, without reference to the value in the controls, is plotted against choline chloride dosage.

The curves indicate that at the higher dosage levels the effect of increasing the concentration of choline chloride in the diet is slight and only at levels of less than 0.05%, under the conditions described, is there a marked correlation between concentration and action. It would appear evident that in assaying the activity of any lipotropic substance it is important to use relatively small doses in order to obtain the most significant relation between the size of the dose and the lipotropic action produced.

It will be appreciated that under any conditions the amount of "extra fat" actually "transported" or otherwise disposed of due to the influence of added choline chloride is relatively small. A 20 g mouse under the experimental conditions will deposit only about 0.15 ± 0.05 g of "extra fat" in the liver in the absence of added choline chloride, although consuming nearly 5 g of fat during the 6-day test period. From the figures it is evident that the smaller the dose of choline chloride the greater is its relative efficiency in preventing the deposition of a portion of this small amount of fat, that is, more fat is "transported" per molecule of choline chloride.

Summary. A 6-day method for the assay of lipotropic substances in the mouse is described. It is shown that a significant relation between the concentration of choline chloride added to a low choline-high fat diet and the lipotropic effect produced exists only at levels approaching the minimum effective dose, under the experimental conditions described.

10071 P

Lipotropic Action of Certain Compounds Related to Choline Chloride.*

Arnold DeM. Welch and Mary Scott Welch. (Introduced by C. F. Cori.)

From the Department of Pharmacology, Washington University School of Medicine, St. Louis.

The hypothesis that choline chloride functions lipotropically by

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* This investigation has been made with the assistance of a grant from the Committee on Therapeutic Research, Council on Pharmacy and Chemistry, American Medical Association. The authors wish to acknowledge their appreciation of the interest and suggestions of Prof. C. F. Cori.

virtue of supplying an essential component of lecithin and possibly other lipid complexes appears particularly worthy of consideration. In this connection many compounds related to choline chloride have been subjected to examination, using the mouse method described in the previous paper. Two of these compounds, the arsenic analogue of betaine hydrochloride and α-methyl-β-phenylcholine chloride have apparently not been prepared hitherto; the preparation of these and other compounds will be described in a later publication. It is desired to list the results obtained with the various substances but to reserve the larger part of the interpretation at present.

TABLE I.

Compound	Formula	Lipotropic Activity
(1) Choline Chloride	$(CH_3)_3N.CH_2CH_2OH$ (Cl)	+
(2) Betaine Aldehyde Chloride[*][‡]	$(CH_3)_3N.CH_2CHO$ (Cl)	+
(3) Betaine Hydrochloride	$(CH_3)_3N.CH_2COOH$ (Cl)	+
(4) Phosphocholine Chloride[‡]	$(CH_3)_3P.CH_2CH_2OH$ (Cl)	+
(5) Phosphobetaine Hydrochloride[‡]	$(CH_3)_3P.CH_2COOH$ (Cl)	—
(6) Arsenocholine Chloride[†]	$(CH_3)_3As.CH_2CH_2OH$ (Cl)	+
(7) Arsenobetaine Hydrochloride[‡]	$(CH_3)_3As.CH_2COOH$ (Cl)	—
(8) β-Methylcholine Chloride[*]	$(CH_3)_3N.CH_2CH(CH_3)OH$ (Cl)	—
(9) α-Methyl-β-phenylcholine Chloride[‡]	$(CH_3)_3N.CH(CH_3)CH(C_6H_5)OH$ (Cl)	?
(10) Ethyl ether-β-methylcholine Chloride[*]	$(CH_3)_3N.CH_2CH(CH_3)O.C_2H_5$ (Cl)	—
(11) Betaine Aldehyde Acetal Chloride[*][‡]	$(CH_3)_3N.CH_2CH(OC_2H_5)_2$ (Cl)	+
(12) α-Methylbetaine Hydrochloride[‡]	$(CH_3)_3N.CH(CH_3)COOH$ (Cl)	+
(13) Calcium Phosphorylcholine Chloride[‡]	$(CH_3)_3N.CH_2CH_2O.PO_3Ca$ (Cl)	+
(14) Trimethylamine Oxide Hydrochloride[‡]	$(CH_3)_3N.OH$ (Cl)	—

[*] Compounds or precursors generously supplied by Merck and Co., Inc.
[†] Generously supplied by Hoffmann-LaRoche, Inc.
[‡] Prepared by the authors.

The writers believe that statements concerning the precise activity of various compounds in terms of choline chloride are unwise unless a large number of experiments have been done in that dosage range where the relation between size of dose and lipotropic effect is most significant. Preliminary observations indicate, however, that N-betaine aldehyde chloride, N-betaine hydrochloride, and the P and As analogues of choline chloride are more than one-half as active as choline chloride.

It will be noted that the P and As betaines are not lipotropic in action. This indicates that the P and As analogues of choline chloride act *per se* (or as the corresponding aldehydes) and suggests, therefore, that N-betaine hydrochloride acts by virtue of its conversion by the organism into choline chloride (or the corresponding aldehyde) and not *per se*.

The testing of the As analogue of betaine aldehyde chloride would appear to be the next step in the investigation of the mechanism of action of choline chloride. The synthesis of this compound will be attempted in the near future. Other lines of investigation now in progress have suggested, however, that the oxidation of choline chloride to betaine aldehyde and betaine[1] which occurs in the liver and kidney of certain species (rat;[2] mouse and dog, recent observations of the writers) is probably not directly concerned with the mechanism by which choline chloride and its related compounds influence the deposition of fat in the liver.

The hypothesis that choline chloride is phosphorylated and utilized in the synthesis of lecithin is supported by the finding that the phosphoric acid ester of choline chloride is unaffected by liver phosphatases and therefore protected from oxidation in the liver. Further, the observations previously described (Welch[3]) concerning the appearance of arsenic in lecithin isolated from rats fed arsenocholine chloride have been confirmed in mice. Semi-quantitative spectrographic analysis of lecithin isolated from mice fed the arsenic analogue for a 6-day period indicates that arsenic is present corresponding roughly to 2% "arsenolecithin." Fractionation and chemical examination of the isolated lecithin are now under way. No arsenic has been found spectrographically in phospholipids of control animals, with or without the addition to the diet of arsenic pentoxide; however, other arsenic compounds will be examined in this regard.

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[1] Quastel, J. H., and Mann, P. J. G., *Biochem. J.*, 1937, **31**, 865; Bernheim, F., and Bernheim, M. L. C., *Am. J. Physiol.*, 1938, **121**, 55.

[2] Welch, M. S., Irving, L., and Best, C. H., *Proc. Am. J. Physiol.*, 1935, **113**, 136; Welch, M. S., and Irving, L., *Proc. Am. J. Physiol.*, 1936, **116**, 159; Trowell, O. A., *J. Physiol.*, 1935, **85**, 356.

[3] Welch, A. DeM., PROC. SOC. EXP. BIOL. AND MED., 1936, **35**, 107.

10072

Vagal Influence on Reflexes of Somatic Musculature.*

William Kaufman. (Introduced by Robert Gesell.)

From the Department of Physiology, University of Michigan, Ann Arbor, Michigan.

Johnson and Luckhardt,[1] King, Blair, and Garrey,[2] and Schweitzer and Wright[3] demonstrated that stimulation of the central vagal stumps inhibits reflex activity of the knee jerk and that, in general, this reflex inhibition is independent of secondary circulatory and respiratory changes incident to central vagal stimulation. It was thought desirable to investigate the effects of interrupting the normal vagal afferent stream upon reflex contractions of the tibialis anticus muscle.

Dogs anesthetized deeply with morphine and urethane were used as experimental animals. The vago-sympathetic trunks were isolated for cold block. The trachea was directly connected to rebreathing tanks designed to absorb carbon dioxide; oxygen was added as necessary. Respiration was recorded by a spirometer directly connected with the rebreathing tanks. Blood pressure was recorded by a mercury manometer from the right femoral artery. The left sciatic nerve was isolated in the thigh; the hamstring branches cut, the posterior tibial nerve identified, severed, and the central end placed on shielded electrodes of special design. The electrodes were connected to the secondary coil of an inductorium whose primary received the discharge from a Thyraton stimulator. After the left leg was immobilized, the tendon of the tibialis anticus muscle was freed for a distance of about 6 cm above its insertion and connected to an isotonic spring tension myograph. A slight constant tension was exerted on the muscle throughout the experiment.

The vagi were cold blocked and recordable reflex contractions were elicited from the tibialis anticus muscle at a constant rate (40 to 60 stimuli per minute). Upon deblocking the vagi, the reflex amplitude gradually decreased to zero, indicating inhibition of the tibialis anticus reflex; upon blocking of the vagi, the reflex contractions of the tibialis anticus muscle gradually reappeared. In some

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* These experiments were supported in part by a grant from the Rockefeller Foundation to Robert Gesell for studies on respiration.

[1] Johnson, C. A., and Luckhardt, A. B., *Am. J. Physiol.*, 1927, **83**, 642.

[2] King, G. E., Blair, E., and Garrey, W. E., *Am. J. Physiol.*, 1931, **97**, 329.

[3] Schweitzer, A., and Wright, S., *J. Physiol.*, 1937, **88**, 459.

animals, this type of alternation was "inexhaustible"; *i. e.,* it could be demonstrated indefinitely by alternate blocking and deblocking of the vagi. In some animals "exhaustible" alternation only could be elicited; *i. e.,* after a short period of vagal blocking and deblocking the reflex arc involved would "fatigue" and only after a period of rest would the alternate inhibition of the somatic reflex be elicited by alternate deblocking of the vagi. In some animals the vagal influence on the reflex contractions of the tibialis anticus muscle could not be demonstrated at any time.

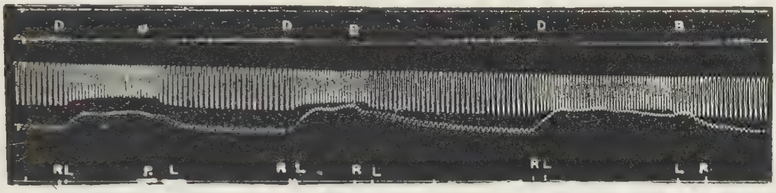

FIG. 1.

Recordings from above downward:
(1) Time
(2) Reflex contraction of the M. tibialis anticus
(3) Respiration
(4) Blood pressure
(5) Signal

Legend:
D = Vagi deblocked
B = Vagi blocked
R = Right vagus
L = Left vagus

As indirect evidence of the tonic inhibitor action of the normal vagal afferent stream, it has been shown (King, *et al.,*[2] and Kaufman[4]) that where respiratory rhythms are evident in continuously elicited reflex contractions, vagotomy or vagal block accentuates these respiratory rhythms.

In a previous paper (Kaufman[4]) it has been shown that the blood pressure changes incident to alternate vagal blocking and deblocking in animals deeply anesthetized with morphine-urethane have no effect directly or reflexly through the pressoreceptor mechanism on the reflex contractions of the tibialis anticus muscle.

It is concluded that the normal afferent vagal stream can reflexly inhibit reflexes of somatic musculature and that the degree of this inhibition varies from animal to animal.

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[4] Kaufman, W., in press.

10073

Sulfanilamide Therapy of Friedländer-Bacillus Infections of Mice.

PAUL GROSS, FRANK B. COOPER AND MARION LEWIS.

From the Western Pennsylvania Hospital Institute of Pathology, Pittsburgh, Pa.

The efficacy of sulfonamide therapy against Friedländer bacillus infections in mice is still open to question. Levaditi and Vaisman[1] failed to demonstrate protection with Prontosil I* and Bürgers[2] was similarly unsuccessful with Prontosil II.† On the other hand, Buttle, *et al.*,[3] claimed "some degree of protection" for sulfanilamide therapy against multiple lethal doses as indicated by an increase in survival time, and several 12-day cures in mice infected with approximately 1 to 10 lethal doses.

The results obtained in a reinvestigation of this problem with 2 virulent strains of Friedländer bacillus are given in Table I. They indicate no significant therapeutic effect of sulfanilamide in mice infected with less than 10 M.L.D.

TABLE I.
Results of Sulfanilamide Therapy in Experimental Friedländer Bacillus Peritonitis of Mice.

Strain	Culture dilution	cc injected intra-abdominally*	No. of mice	Mg daily oral dose of sulfanilamide†	No. of survivors						
					1	2	3	4	5	6-14	(Over 14 days)
Tannehill M	10^{-3}	0.5	10	0	8	–	–	–	–	–	2
,,	10^{-3}	0.5	10	20	8	2	–	–	–	–	0
Tannehill M5	10^{-5}	0.5	10	0	2	3	2	2	–	–	1
,,	10^{-5}	0.5	10	25	1	2	1	2	–	–	4
Williams M4	10^{-4}	0.5	10	0	1	4	2	–	–	2	1
,,	10^{-4}	0.5	10	20	0	3	2	1	1	3	0
,,	10^{-5}	0.5	10	0	1	3	–	1	–	3	2
,,	10^{-5}	0.5	10	20	–	2	–	1	–	3	4

* The amount injected corresponded to less than 10 M.L.D., determined by previous and simultaneous titrations.

† The first dose of sulfanilamide was given 3 hours after the inoculation and every day thereafter for 6 days.

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[1] Levaditi, C., and Vaisman, A., *Compt. rend. Soc. de biol.*, 1935, **119**, 946.

* 2,4-Diaminoazobenzene-4′-sulfonamide.

[2] Bürgers, *Deutsche med. Wchnschr.*, 1937, **63**, 672.

† Disodium 4′-sulfonamidophenyl-2-azo-7-acetylamino-1-hydroxynaphthalene-3,6-disulfonate.

[3] Buttle, G. A. H., Parish, H. J., McLeod, M., and Stephenson, D., *Lancet*, 1937, **1**, 681.

The action of sulfanilamide, 4,4′-di-(acetylamino)-diphenylsulfone, and 4,4′-diaminobenzenesulfonanilide on mice given single lethal doses of killed Friedländer bacilli was also investigated. The experimental set-up and results are given in Table II, in which the absence of any appreciable therapeutic effect of sulfanilamide is shown. The other 2 drugs were also inactive (Table II). These results are similar to those obtained in experiments with killed meningococci and streptococci, which are reported elsewhere.[4]

TABLE II.
Results of Chemotherapy in Mice Given Lethal Doses of Killed Friedländer Bacillus.

cc injected intraäbdominally*	No. of mice	Mg daily oral dose of drug†			No. of mice dead in days			No. of survivors (over 6 days)
		A	B	C	1	2	3	
.15	10	—	—	—	6	1	1	2
.15	10	15	—	—	1	6	2	1
.20	10	—	—	—	9	1	–	0
.20	10	25	—	—	3	2	2	3
.20	10	—	25	—	9	1	–	0
.20	10	—	—	25	7	2	–	1

* Heavy suspension of formalin-killed Friedländer bacilli (washed free of formalin and tested for sterility) adjusted so that 0.20 cc equals 1 M.L.D.

† The first dose of drug was given one hour prior to the injection of organisms.

A—sulfanilamide.
B—4,4′-di-(acetylamino)-diphenylsulfone.
C—4,4′-diaminobenzenesulfonanilide.

Conclusions. Sulfanilamide has no significant therapeutic activity in mice infected with less than 10 M.L.D. of 2 strains of Friedländer bacillus.

No therapeutic effect was observed with sulfanilamide, 4,4′-di-(acetylamino)-diphenylsulfone, or 4,4′-diaminobenzenesulfonanilide in mice injected with one M.L.D. of killed Friedländer bacilli.

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[4] Gross, P., Cooper, F. B., and Lewis, M., *J. Inf. Dis.*, in press.

10074

Distribution of Glycogen in the Dog's Heart.*

ROBERT W. BOYLE AND C. H. McDONALD. (Introduced by Paul L. Day.)

From the Department of Physiology and Pharmacology, the University of Arkansas School of Medicine, Little Rock.

Dogs free of disease and in a good state of nutrition were anesthetized by the intravenous injection of sodium phenobarbital solution (50 mg to the cc). From 100 to 150 mg per kilo induced surgical anesthesia in from 4 to 5 minutes. The chest was quickly opened with scissors and bone forceps, the heart removed while still beating and divided into the 2 atria, the 2 ventricles, and the septum. A piece of each was excised, dropped into a weighed hydrolyzing tube, and weighed. A hot hydrolyzing fluid (30% KOH) was then added. The whole procedure from opening the chest to this point consumed less than 5 minutes.

Hydrolysis of the tissue was carried out according to Pflüger's method as modified by Good, Kramer, and Somogyi.[1] We made 2 further slight modifications: (a) The tissue was weighed before the hydrolyzing fluid was added and (b) following acid hydrolysis of the glycogen the solution was made neutral to litmus by the addition of NaOH solution. Glucose was determined by the method described

TABLE I.
Glycogen in Normal Dog Hearts (mg per g of tissue calculated as glucose).

	Atria		Ventricles		
No.	Right	Left	Right	Left	Septum
1	4.44	5.53	6.98	4.29	6.63
2	6.67	9.37	10.42	6.71	7.15
3	5.50	8.29	12.44	9.28	10.16
4	5.60	6.84	7.78	6.57	5.98
5	6.13	7.42	6.95	5.75	4.85
6	5.90	8.89	10.33	7.50	7.19
7	5.27	5.05	5.82	4.30	4.70
8	4.65	5.01	6.03	6.81	6.63
9	5.85	8.44	10.15	5.87	5.88
10	3.45	8.02	4.16	4.38	4.35
11	6.76	8.03	12.94	8.13	10.68
12	6.58	11.14	12.58	10.80	7.44
Avg.	5.57	7.67	8.88	6.70	6.80

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* Research paper No. 514, Journal Series, University of Arkansas.

[1] Good, C. A., Kramer, H., and Somogyi, Michael, *J. Biol. Chem.*, 1933, **100**, 485.

by Shaffer and Somogyi,[2] using the modified reagent No. 50 and the modified procedure suggested by Somogyi.[3] Table I contains the results of these analyses.

Summary. While individual hearts show wide variations in the comparative contents of glycogen in the several parts, averages for the 12 hearts studied indicate that in a large series these differences would not be great. In general, the left atrium and the right ventricle show slightly higher percentages than do the remaining parts of the heart. Whether this greater content would hold for a larger series and possible structural or functional significance of such differences are matters entirely speculative in nature.

10075 P

Effect of Parathyroid Hormone on Diffusion of Calcium, Magnesium and Phosphorus into the Peritoneum. I.

A. Cantarow, Victor G. Haury and C. G. Whitbeck.

From the Departments of Medicine and Pharmacology, Jefferson Medical College, and the Laboratory of Biochemistry, Jefferson Hospital, Philadelphia.

Eight adult dogs were given intraperitoneal injections of 2.5% dextrose and 0.9% NaCl solution in the dosage of 100 cc per kg. After 16 hours, 20 cc of blood were withdrawn from the femoral artery and 20 cc of fluid from the peritoneum. Five hundred units of parathyroid hormone* were then injected intramuscularly. Similar blood and fluid samples were obtained hourly for 8 hours and were examined for Ca, Mg and inorganic P content.

Magnesium. The control values for serum Mg were 2.1-3.26 mg %. In 6 cases parathyroid administration was followed by a fall in serum Mg, the maximum decrease being 0.35-0.8 mg % at 1-6 hours, with a tendency to return to normal at 3-8 hours. In one case the serum Mg increased (0.3 mg). No significant change occurred in one case.

The control values for peritoneal fluid Mg were 1.75-2.0 mg %. These increased in each instance, the maximum increase being 0.17-1.02 mg %, the peak occurring at 5-8 hours. The control ratio of fluid to serum Mg was 57-87%, the mean being 75%. This ratio rose in each case, the maximum rise being 12-46% at 5-7 hours.

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[2] Shaffer, Phillip A., and Somogyi, Michael, *J. Biol. Chem.*, 1933, **100**, 695.

[3] Somogyi, Michael, *J. Biol. Chem.*, 1937, **117**, 771.

* Aided by a grant from Parke, Davis and Co.

The relatively slight changes in serum Mg after parathyroid hormone administration are in accord with previous reports.[1, 2, 3] However, these described a primary increase in serum Mg followed by a decrease, whereas the opposite occurred in the majority of animals in the present series. The most consistent change was an increase in the amount of diffused Mg, suggesting that the parathyroid hormone increases the diffusibility of serum Mg.

Inorganic P. Control values for serum P were 3.1-5.4 mg %. These fell in every case after parathyroid administration, the maximum decrease being 0.6-1.1 mg. The lowest values occurred at 1 hour in one case, at 3 hours in 3, at 4 hours in 2 and at 5 and 6 hours in one case each, with a subsequent rise in each instance.

The control values for peritoneal fluid P were 2.6-5.0 mg %. These fell in 5 cases, the maximum decrease being 0.6-1.2 mg %. The lowest values occurred at 4 hours in 2 cases and at 6 hours in 3, with a subsequent rise. In 2 cases the fluid P increased progressively, the maximum rise being 0.8 mg and 1.7 mg %, at 7 and 6 hours respectively. No significant change occurred in one case. The control ratio of peritoneal fluid to serum P was 83-95%. This increased in 6 cases, the maximum rise being 10-19% at 2-5 hours, with a subsequent fall. It decreased in 2 cases, the maximum fall being 11 and 12%, at 6 and 4 hours, with a subsequent rise.

The changes in serum P are in accord with previous reports. The fact that the serum and fluid P concentrations diminished practically simultaneously in 5 cases suggests the existence of a rather continuous diffusion equilibrium between the fluid and blood plasma. The establishment of this point would be of great importance in connection with the interpretation of the concentrations of Ca, Mg and P in the fluid as indicative of the level of the diffusible fractions of these elements in the plasma.

Calcium. The control serum calcium values were 9.8-11.2 mg %. The maximum increase was 1.8-2.7 mg, the peak occurring at 5-6 hours with a subsequent slight decline. The control values for peritoneal fluid Ca were 5.1-7.0 mg %. This rose in each case, the maximum increase being 1.7-2.7 mg %, the peak occurring at 8 hours in 7 cases and at 6 hours in one case.

The control ratios of fluid to serum Ca were 52-69%, the mean being 61%. No significant change occurred in the ratio for about 5 hours; it then increased to a maximum of 63-86% at 8 hours (end of experimental period). The degree of increase was 11-24%.

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[1] Scholtz, H. G., *Arch. exp. Path. u. Pharmakol.*, 1931, **159**, 233.

[2] Greenberg, D. M., and Mackey, M. A., *J. Biol. Chem.*, 1932, **98**, 765.

[3] Bulger, H. A., and Gausmann, F., *J. Clin. Invest.*, 1933, **12**, 1135.

In only one case was the period of increasing calcemia accompanied by an increased fluid-serum Ca ratio. In 5 cases the ratio had returned to the control level after 24 hours, as had the fluid and serum Ca concentrations. The protein concentration of the peritoneal fluid remained practically constant throughout the experimental period (0.25-0.6%).

The question arises as to whether the concentration of Ca in the peritoneal fluid is representative of the level of the diffusible fraction of serum Ca at any particular time. Such an assumption presupposes the existence of a constant and rapidly established equilibrium between the plasma and fluid. No evidence is available bearing directly on this point. However, certain related observations are possibly of significance in this connection. In 4 animals, 50 cc of fluid were injected intraperitoneally instead of 100 cc. The findings were essentially the same as presented above. In other experiments, increase and decrease of the serum Ca were accompanied by corresponding changes in the fluid Ca, suggesting a rapid establishment of equilibrium between the two media. This is also suggested by the findings with regard to changes in P, as noted above.

There is evidence, therefore, that the equilibrium between the serum and peritoneal fluid Ca is established rapidly in the face of moderate changes in the concentration of this element in the serum. If the fluid Ca concentration may be regarded as representing the level of diffusible serum Ca, it appears that parathyroid hypercalcemia, under the conditions of the experiment, is due to a proportional increase in diffusible and nondiffusible fractions of serum calcium. This is in accord with findings obtained in artificial membrane experiments. It appears, however, that the increase in the diffusible fraction persists or continues for some hours after the nondiffusible fraction has begun to diminish, the normal ratio being restored before 24 hours. Whether or not the peritoneal fluid Ca is truly representative of the level of diffusible serum Ca, it seems logical to assume that it does represent the level of Ca in the tissue fluids throughout the body, *i. e.*, "diffused" Ca. These findings may, therefore, give more exact information regarding the physiologic effect of the parathyroid hormone than can be obtained by serum Ca determinations or studies of serum Ca diffusibility by artificial membrane methods.

10076 P

Effect of Parathyroid Hormone on Diffusion of Calcium into the Peritoneum. II.

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From the Departments of Medicine and Pharmacology, Jefferson Medical College, and the Laboratory of Biochemistry, Jefferson Hospital, Philadelphia.

Two adult dogs, which had been subjected to repeated (7 times each) filling and emptying of the peritoneal space (dextose, 2.5%-NaCl, 0.9% solution, 100 cc per kg) during the preceding 3 weeks, were treated as indicated in Fig. 1. The purpose of the repeated filling and evacuation of the peritoneum, particularly during the 24 hours preceding the injection of parathyroid hormone, was to determine whether the removal, by this means, of diffusible calcium

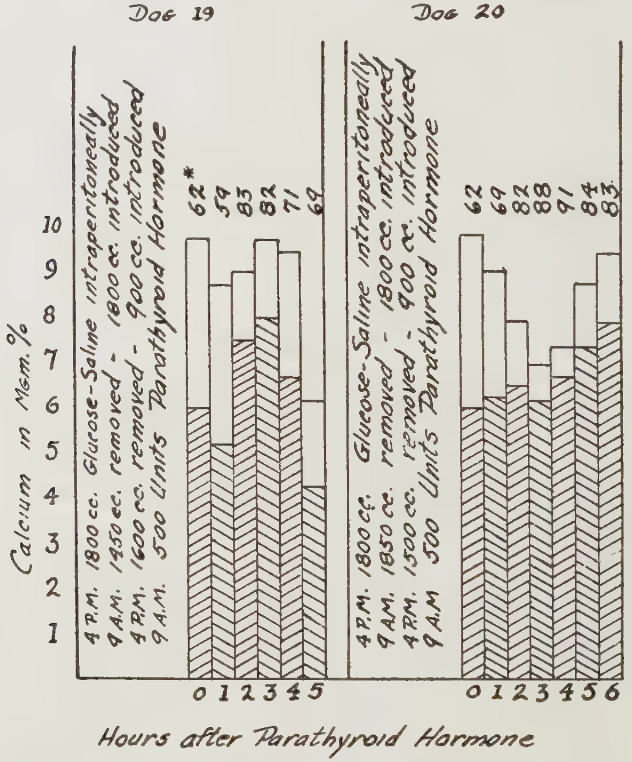

FIG. 1.

* Ratio (%) of peritoneal fluid calcium to total serum calcium concentration.

from the blood would influence the response to parathyroid hormone.*

In Dog 19 (18 kg), a total of 183 mg, and in Dog 20 (18 kg), 201 mg of calcium was removed in the peritoneal fluid in the 24 hours immediately preceding the intramuscular injection of parathyroid hormone. No food and only distilled water were allowed during the experimental period.

Three points of interest are revealed by these data.

(1) Under certain conditions, the administration of parathyroid hormone may result in hypocalcemia. That these animals were not refractory to the hormone was indicated by the fact that changes occurred in the serum and peritoneal fluid magnesium which were identical with those observed in a series of cases in which hypercalcemia was produced by the hormone.[1]

(2) A fall in serum calcium may be accompanied by a fall in the peritoneal fluid calcium. This is of importance in view of the doubt as to whether the concentration of calcium in the fluid is capable of changing, and especially of decreasing rapidly simultaneously with corresponding changes in the diffusible fraction of serum calcium.

(3) An actual and significant increase in "diffused" calcium may occur following parathyroid hormone administration with a constant (Dog 19) or a falling (Dog 20) serum calcium concentration.

These findings suggest that the primary effect of the parathyroid hormone on serum calcium is to increase its diffusion from the blood into the tissue fluids. If the peritoneal fluid calcium concentration may be regarded as representing the level of the diffusible serum calcium fraction, the primary effect of the hormone on serum calcium appears to be to increase this fraction regardless of the occurrence or direction of simultaneous changes in the total serum calcium concentration.

Under normal conditions, this increase is believed to be the result of active mobilization of calcium from the bones, with consequent hypercalcemia. Under such circumstances the maintenance of the normal ratio of diffusible to total serum calcium is due perhaps to prompt "binding" of some of the mobilized calcium by serum protein or in a "colloidal calcium phosphate complex,"[2] masking the primary nature of the increase in the diffusible fraction. The fact that the peritoneal fluid-serum calcium ratio increased to 83% and 91% with a constant or a falling serum calcium concentration suggests the possibility that the parathyroid hormone may increase the diffusible calcium at the expense of the nondiffusible serum calcium fraction under certain conditions. Why the production of these phenomena should be effected by the removal from the organism of such relatively small amounts of calcium as 183 and 201 mg under the conditions of the experiment cannot be stated at the present time.

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* The parathyroid hormone was supplied by Dr. E. A. Sharp, of Parke, Davis and Company. Aided by a grant from Parke, Davis and Company.

[1] Cantarow, A., Haury, V. G., and Whitbeck, C. G., PROC. SOC. EXP. BIOL. AND MED., 1938, **39**, 15.

[2] Cantarow, A., Brundage, J. T., and Housel, E. L., *Endocrinology*, 1937, **21**, 368.

10077

Influence of Testosterone Propionate on Vaginal Opening in the Immature Albino Rat.*

H. S. RUBINSTEIN, A. R. ABARBANEL AND D. N. NADER. (Introduced by J. C. Krantz, Jr.)

From the Research Laboratory, Surgical Division, Sinai Hospital, Baltimore, Md.

Butenandt and Kudszus[1] observed that androgenic substances administered to immature female rats produced premature vaginal opening. Since then Salmon[2] has attempted to determine whether this effect (vaginal opening) was the result of a direct action of the androgenic substance upon the genitals or whether the effect was secondary to a stimulating action of the androgen on the ovary. Because Salmon observed follicle stimulation or luteinization (or both) in his test animals, he concluded that the premature vaginal opening which he noted was due to the effect of the testosterone propionate and androstenediol (which he used) upon the ovary. Since this author did not use spayed animals it is difficult to see how a direct effect of the androgenic substance upon the genital organs can be ruled out. For this reason, the following experiments which utilized both non-castrate and castrate animals as test objects are reported.

Fifty-two female albino rats *(Mus norvegicus*—var. *alba)* of Wistar Institute stock 26 days of age were divided into 4 groups consisting of (1) 16 non-spayed test animals; (2) 10 spayed litter-mate test animals; (3) 16 non-spayed litter-mate controls; and (4) 10 spayed litter-mate controls. Castrations were carried out at 22 days of age. The test animals were treated with injections of testosterone propionate (Perandren-Ciba) in a dosage of one mg daily (except Sunday) which were administered intraperitoneally beginning on the 26th day of age. The control rats remained uninjected. All animals were maintained under similar environmental conditions, being fed on purina dog chow (cubes) daily and green vegetables twice weekly. Water was constantly present.

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* The authors gratefully acknowledge the cooperation of the Ciba Pharmaceutical Products, Inc., who partially defrayed the expenses involved in this study.

[1] Butenandt, A., and Kudszus, H., *Hoppe-Seyler's Z. Physiol. Chem.*, 1935, **237**, 75.

[2] Salmon, U. J., PROC. SOC. EXP. BIOL. AND MED., 1936, **35**, 49.

Daily observations of all animals disclosed that vaginal opening occurred in the treated normal animals at 31 to 35 days of age, in the treated spayed animals at 32 to 35 days and in the normal controls at 40 to 48 days of age. While some of the spayed controls acquired spontaneous vaginal opening at 65 to 70 days, others failed to show this phenomenon at 75 days of age.

When the means of these observed ages are compared (Table I) it is seen that vaginal opening in the treated non-spayed and spayed animals occurred 11.7 ± 0.54 and 10.5 ± 0.60 days respectively earlier than in the normal controls, while the treated non-spayed animals showed this phenomenon only 1.2 ± 0.41 days sooner than the treated castrate group.

TABLE I.

Showing a comparison between the ages (variation and means) at which vaginal opening was noted. The differences between groups are compared and the ratios between the differences and their respective probable errors listed.

Group	No. of Animals	Age at Time of Vaginal Opening		Difference Noted	Critical Ratio
		Range	Mean±P.E.		
Castrate test	10	32-35	33.8±0.34	1.2±0.41	2.92
Normal test	16	31-35	32.6±0.23		
Normal control	16	40-48	44.3±0.49	11.7±0.54	21.66
” test	16	31-35	32.6±0.23		
Normal control	16	40-48	44.3±0.49	10.5±0.60	17.50
Castrate test	10	32-35	33.8±0.34		

It is interesting to note that vaginal smears made at the time of vaginal opening failed to reveal a full estrus picture in any of the animals examined.

From these results it is seen that testosterone propionate (Perandren-Ciba) had a marked stimulating effect on vaginal opening in both non-spayed and spayed immature animals. That this observation is in all probability highly significant can be appreciated from the high critical ratios (C. R.) noted in the table.

On the other hand, in spite of the fact that the critical ratio between the castrate and non-castrate test animals approaches "3" (actually 2.92 in Table I) which would ordinarily lead one to suspect a probable significance in the difference noted, such significance is questionable for the following reasons. First, it will be noted (Table I) that while the vaginal opening of the treated non-castrate animals occurred in some cases a day earlier than in some of the treated castrate rats, the vaginas of all treated animals were open by the 35th day. Secondly, in some animals not included in the experiments herein reported, vaginal opening under similar conditions was seen to occur on the 30th day of age. Finally, in experiments reported elsewhere[3] it was observed that the Graffian follicle under the influence of chronic injections of testosterone propionate was actually inhibited in growth and failed to ovulate.

Conclusions. Testosterone propionate (Perandren-Ciba) stimulates vaginal opening in both non-spayed and spayed immature female rats. This substance, therefore, does have an effect on the vaginal zone which is independent of the ovary. Because such direct effect has been noted and because the age at which vaginal opening occurs in the non-spayed and spayed test groups corresponds so closely it is believed that the intact ovary plays but a negligible part in the process under the conditions of these experiments.

10078 P

Effect of Sulfanilamide on Experimental Tuberculosis in the Guinea Pig.

P. H. GREEY, H. H. CAMPBELL AND A. W. CULLEY. (Introduced by W. L. Holman.)

From the Department of Bacteriology, University of Toronto, and the Toronto General Hospital.

Sulfanilamide has been found by Rich and Follis[1] to exert a markedly inhibitory effect upon the development of experimental tuberculosis in the guinea pig. These authors noted that 100 mg of sulfanilamide in a single daily dose did not modify the tuberculous process, while 2 daily doses of 100 mg produced considerable inhibition. This was much more marked when 500 mg of sulfanilamide, divided into 4 doses, were given daily. In their experiments treatment was commenced 3 days before the subcutaneous injection of the human tubercle bacilli. Smithburn,[2] on the other hand, failed to find any beneficial effect from the daily administration of 200 mg of sulfanilamide intraäbdominally to guinea pigs that had been infected 24 hours before by the intracerebral inoculation of a small number of virulent human tubercle bacilli.

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[3] Rubinstein, H. S., *J. A. M. A.*, in press.

[1] Rich, A. R., and Follis, R. H., Jr., *Bull. Johns Hopkins Hosp.*, 1938, **62**, 77.

[2] Smithburn, K. C., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 574.

The present report deals with the effect of sulfanilamide upon the tuberculous process in the guinea pig before, and at different intervals after, infection. Forty-eight guinea pigs averaging about 350 g were used in groups of 12. The drug (Prontylin powder, Winthrop Chemical Company) was administered *per os* throughout the experiment in measured doses of 100 mg at 9 a.m., 5 p.m., and 11 p.m. daily, in powdered form with the aid of a nasal speculum, and the animals were found to retain practically all of the drug with this technic. These guinea pigs were infected by the subcutaneous injection of 0.2 cc of a suspension of virulent human tubercle bacilli containing 2 to 6 microörganisms per oil-immersion field. The 12 guinea pigs in group A received sulfanilamide 3 days before, those in group B 17 days after, and in group C 24 days after the infecting injection. At death the treated animals were necropsied and the macroscopic and microscopic findings were compared with those of the control animals that had been infected for a similar period of time. This was made possible by using as controls the animals of groups B and C that died before treatment.

The lesions in the spleens and livers of the animals that had received sulfanilamide before infection were small and few in number in contrast to the many prominent tubercles in the control animals. This difference was even more marked microscopically and but few acid-fast bacilli were found in the lesions compared to those of the controls. The inhibition of the tuberculous process in these organs by sulfanilamide, however, was not quite as marked as that described by Rich and Follis. Furthermore, the extent of the local lesion at the site of the injection was not greatly modified by treatment in this group. In the 2 groups that received treatment 17 and 24 days respectively after infection, there was no appreciable difference noted in the amount or extent of macroscopic tuberculosis. But microscopically the lesions in the treated animals usually could be distinguished from those of the control group in that they presented less extensive necrosis and greater proliferation of endothelial cells.

All of the animals used in these experiments were tested intracutaneously by 0.1 cc of old tuberculin at varying intervals during the infection. The development of sensitivity was not significantly affected in any way by the drug.

The daily administration of 300 mg of sulfanilamide was found to be definitely toxic for the infected guinea pig. This was shown by the fact that the control animals survived longer than those treated. The toxicity of the compound was greatest for pure-white guinea pigs.

Conclusions. Under the conditions of the experiment it was found that sulfanilamide had an inhibitory effect upon the development of tuberculosis in the guinea pig if the drug was administered before the infection was given. When the infection had been present 17 to 24 days, treatment with sulfanilamide did not alter the macroscopic appearance of the tuberculous process. The development of sensitivity to tuberculin was unaltered by sulfanilamide therapy.

10079

Analysis of Insulin Response of Rabbits After Injection of Diabetic Serum.

F. C. DOHAN. (Introduced by F. D. W. Lukens.)

From the George S. Cox Medical Research Institute, University of Pennsylvania, Philadelphia.

Since the measurement of substances antagonistic to insulin is not possible by direct chemical methods, various indirect physiological methods have been tried. For this purpose De Wesselow and Griffiths[1] used the response of rabbits to insulin before and after the injection of plasma from diabetic patients. Their results suggested that insulin-sensitive and insulin-insensitive types of diabetes might be differentiated in this manner. Our efforts to use this method have led to inconclusive results. An analysis of these results is presented which illustrates the difficulties in interpreting this test. It is probable that equal caution is necessary in appraising any other test where the response to insulin is used. For purposes of comparison the diminution in the response to insulin produced by pituitary extracts has been included.

The fall in the blood sugar of rabbits after insulin was observed before and after the injection of serum from diabetic patients. The test was carried out as described by De Wesselow,[1] except that 10 cc of fresh serum was used instead of 10 cc of plasma. Three hours after the subcutaneous injection of the serum, the fasting blood sugar was taken. Insulin (0.2 unit per kg) was then given intravenously and the blood sugar observed every 15 minutes for one hour. The difference between the initial blood sugar and the lowest blood sugar obtained during the test has been expressed as the percent fall from the initial level.

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[1] De Wesselow, O. L. V., and Griffiths, W. J., *Lancet*, 1936, **230**, 991.

The alteration of the insulin response in rabbits after the injection of crude saline extract of anterior pituitary (beef) was observed. The pituitary extract was injected in doses ranging from the equivalent of 1.5 g of fresh anterior pituitary given 3 hours before insulin to doses several times this amount given daily from 3 to 5 days. In a few of the experiments with daily injections no extract was given

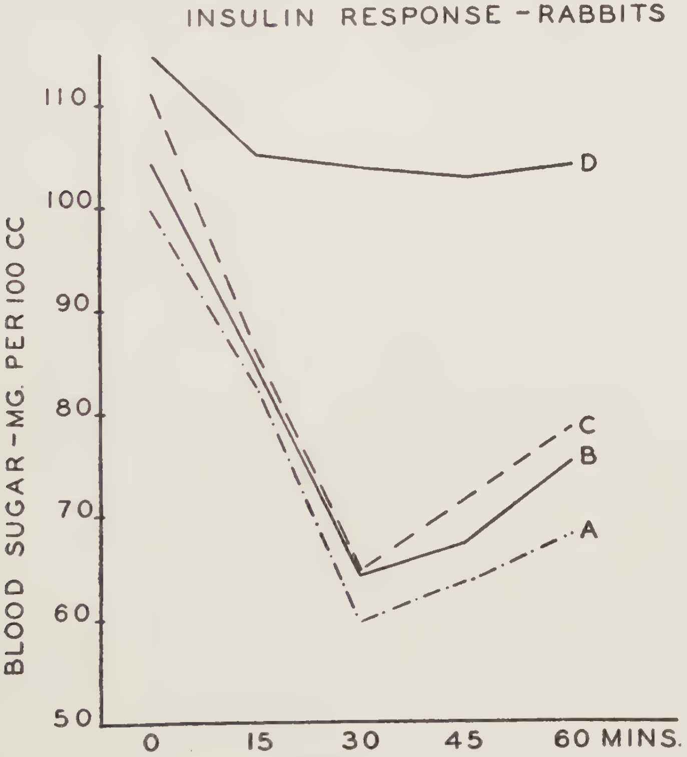

FIG. 1.

The effect of diabetic serum and anterior pituitary extract on response to insulin in the rabbit.

A. Average of 48 control tests. Insulin given intravenously in dose of 0.2 units per kg.

B. Average of 20 tests after the injection of serum from diabetics over 49 years old.

C. Average of 14 tests after the injection of serum from diabetics under 49 years old.

D. Average of 14 tests after the injection of anterior pituitary extract.

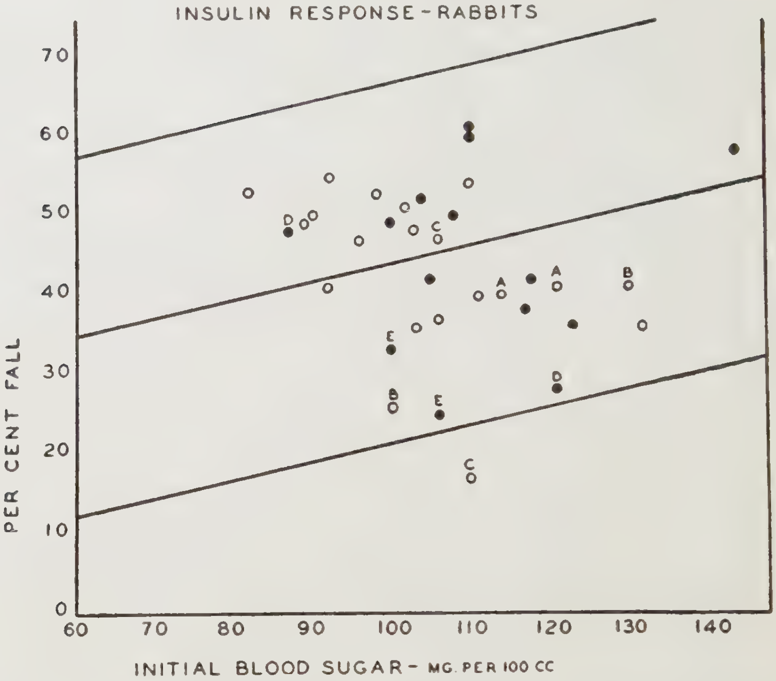

Fig. 2.

○ Injected with serum of diabetics over 49. A, B, C—each tested twice with same sera.

● Injected with serum of diabetics under 49. D, E—each tested twice with same sera.

Middle line is regression line of 48 control rabbits injected with insulin only.

Upper and lower lines indicate twice the standard deviation of the control series.

on the day of the insulin test. Insulin (0.2 units per kg) was given intravenously and blood sugars taken at 15-minute intervals as in the animals given diabetic serum. In all, 14 tests were performed.

The results of 48 insulin tests in 40 normal rabbits were analyzed. The mean initial blood sugar was 100 mg per 100 cc and the average fall was 44% of initial value. The percent fall increased with the increase in the initial blood sugar level. This finding is statistically significant and is in close agreement with the results obtained by Hemmingsen and Marks[2] in 2900 rabbits. The standard deviation of the percentile falls in our small series was ± 11.4, a figure comparable to that in Culhane's[3] series of more than 1500 tests. In the few of our rabbits in which repeated tests were performed there was

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[2] Hemmingsen, A. M., and Marks, H. P., *Quart. J. Pharm.*, 1932, **5**, 245.

[3] Culhane, K., *Quart. J. Pharm.*, 1928, **1**, 517.

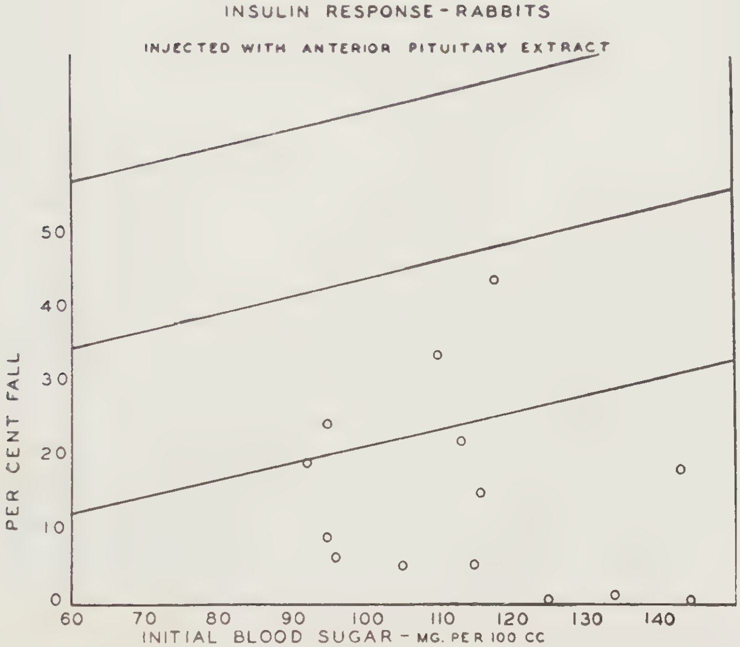

FIG. 3.

○ Response to insulin of rabbits after injection of anterior pituitary extract. The lines have the same significance as in Fig. 2.

a wide range of response in spite of the care taken to make the tests uniform.

The results of 34 insulin tests in rabbits previously injected with serum from diabetic patients are shown in Figs. 1 and 2. Although the curves (Fig. 1) show a slight difference between the normal and the injected rabbits this apparently is not significant when the results are further analyzed (Fig. 2). An arbitrary age grouping has been made in Figs. 1 and 2, since De Wesselow found that insulin-insensitive diabetics were more numerous among older patients. In this series the separation of juvenile diabetics did not reveal any greater difference than that shown in Fig. 1 where the age 50 was selected.

On the other hand a pronounced inhibition of the insulin response was obtained (Fig. 1, curve D, and Fig. 3) following the injection of anterior pituitary extract. It should be emphasized that these results were secured only after obtaining pituitaries frozen on removal from the steer. Before this was done 18 tests, using various crude and commercial extracts, were performed. Of these only 2 showed the least evidence of suppression of insulin action, a fact which suggests that the non-specific effect of tissue injections is not responsible for the results.

The rabbit test proposed by De Wesselow and Griffiths[1] to differentiate insulin-sensitive and insulin-insensitive diabetics has failed to do this in our hands. While differences in technic may explain this, it appears that there are more fundamental difficulties to be overcome before a clinical assay of contra-insular mechanisms by this method is possible. The nature of these difficulties has been fully discussed by De Wesselow and Griffiths[4] in relation to the use of glucose and insulin in diabetics.

The confirmation of the insulin antagonistic action of whole pituitary extracts in rabbits needs no comment.[5, 6] It is so definite that it suggests a contrast rather than a similarity between these experiments and clinical diabetes.

Summary. The response of rabbits to insulin before and after the injection of diabetic *serum* has not, in our experience, afforded a means of distinguishing different types of diabetes. The inhibition of the response of the blood sugar to insulin in rabbits after the injection of pituitary extract has been confirmed.

10080

Infection with *Trypanosoma lewisi* in the Hypophysectomized Rat.

JAMES T. CULBERTSON AND NORMAN MOLOMUT. (Introduced by F. P. Gay.)

From the Department of Bacteriology, College of Physicians and Surgeons, Columbia University, New York City.

Trypanosoma lewisi is a natural parasite of the blood of wild rats, and can be transferred easily to laboratory rats. After inoculation to normal adult rats, it produces a blood stream infection of considerable intensity, although only rarely does the infected animal die. At the end of a patent period ranging from a few day to several weeks, the animals usually clear themselves of the infection and thereafter wholly resist reinfection with this parasite.[1] In the present paper are reported the results of infecting hypophysectomized rats with *Trypanosoma lewisi.* As will be seen, the hypophysectomized rat dies after infection with this parasite, death occurring in most cases 5 days after the parasite is injected.

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[4] De Wesselow, O. L. V., and Griffiths, W. J., *Quart. J. Med.*, 1938, **7**, 17.

[5] Cope, O., and Marks, H. P., *J. Physiol.*, 1934, **83**, 157.

[6] Young, F. G., *J. Physiol.*, 1936, **87**, 13P.

[1] Taliaferro, W. H. "The Immunology of Parasitic Infections," New York, Century, 1929.

Procedure. The experimental work was designed: (1) to compare infection with *Tryp. lewisi* in hypophysectomized rats with that in control rats,* and (2) to study the effect of the administration of anterior pituitary extract upon the infection in the hypophysectomized rats. Thirty-three rats 60 days old were used for the first purpose of the work, 19 being hypophysectomized† and 14 serving as controls. At infection, all animals were given one million *Tryp. lewisi* intraperitoneally. The hypophysectomized rats, together with 12 of the control rats which were subjected to the identical operative procedure except for the removal of the hypophysis, were infected from 5 to 10 days after operation. The 2 remaining control rats were normal unoperated animals. For the second purpose of the work, 14 hypophysectomized rats, infected 5 days after operation, were treated subcutaneously or intraperitoneally daily with 10 rat units of E. R. Squibb and Sons' anterior pituitary extract‡ beginning the first day after operation and continuing until death or recovery from the infection. It was thought that growth factors in this extract might nullify the effects of the parasite in the hypophysectomized rats. All of the animals used in these experiments were examined practically every day, the parasites being estimated by counts with the hemocytometer or, early in the patent period, by direct observation of a drop of fresh blood with the "high-dry" power (×430) of the microscope.

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* The strain of *Trypanosoma lewisi* was obtained from Dr. L. Reiner, Director of Research, the Burroughs Wellcome and Company, Tuckahoe, New York. The rats were of the Long-Evans strain, bred in this laboratory of stock obtained from Dr. P. E. Smith of the Department of Anatomy of the College of Physicians and Surgeons. They were maintained upon the McCollum diet consisting of ground whole wheat (67.5%), whole milk powder (10%), milk fat (5%), casein (15%), sodium chloride (1%), and calcium carbonate (1.5%).

† The method of hypophysectomy was in principle the parapharyngeal approach of Smith,[2] although the actual operative procedure used was modified from that of Selye.[3] Of over 200 rats operated upon by one of us (N.M.) but not infected with *Trypanosoma lewisi,* death from the operation occurred in only two, and the remainder survived regularly for at least 50 days.

[2] Smith, P. E., *Am. J. Anat.,* 1930, **45**, 205.

[3] Selye, H., *Virchow's Arch.,* 1933, **290**, 23.

‡ Kindly supplied by the endocrine clinic of the Presbyterian Hospital (Dr. R. Kurzrok), New York City.

TABLE I.
Summary of Results of Infecting Hypophysectomized Rats and Control Rats with *Tryp. lewisi.*

Group	No. of rats	Treatment	No. dead	Avg survival time (days) of those dead	Avg *T. lewisi*/mm.^{3} on 4th day of infection
Hypophysectomized	19	None	19	5.4	90,000
Operated controls	12	”	2	7.5	85,000
Unoperated ”	2	”	0	—	65,000
Hypophysectomized	14	Ant. pituitary extract (Squibb)*	8	4.8	107,000

* 10 rat units daily.

Results. All of the 19 hypophysectomized rats given no pituitary extract died after infection with *Tryp. lewisi.* The intensity of infection in these animals did not exceed that in the controls, although death generally came at about the time of the peak of the infection, *i. e.,* 5 days after infection. Two deaths occurred among the 12 operated control animals, although the 10 remaining operated controls as well as 2 unoperated controls survived the infection with no apparent difficulty. The hypophysectomized rats did not suffer an anemia more severe than that generally observed in the normal rat during this infection, and blood films treated with Wright's stain were free of *Bartonella muris.* Death was precipitous and convulsive, resembling that from "pathogenic" trypanosomes in the rat. The attempt to replace the hypophysis by administration of anterior pituitary extract was successful in only 6 of 14 animals, the remaining 8 rats succumbing to the infection. The cause of failure of the extract to protect the rats which died is obscure. However, since endocrine extracts are as yet comparatively crude products, they are sometimes irregular in the effects which they produce. Furthermore, it is possible that the type of extract used was not the most effective one functionally to replace the pituitary under the conditions of this experiment. All animals were autopsied at death; the spleen was invariably enlarged from one to 3 times, and the lungs were invariably slightly or deeply hemorrhagic. In many autopsies upon nonhypophysectomized rats with *Tryp. lewisi,* no such congestion of the lung has been noted in this laboratory.

10081

Early Movements of the Opossum with Special Reference to the Walking Gait.*

G. E. Coghill.

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The specimens from which these records were made were of the first, fourth and seventh day, with an additional litter between the fourth day and the seventh, and one slightly older. In no case was the time of birth observed. The age according to days was determined simply by inspecting the females for the presence of young in the pouch each day about 8 A. M.

The young were forcibly taken from the pouch and immediately placed in an incubator designed for the purpose. It is closed on one side only with a curtain which hangs loosely enough to admit the observer's hands. The top, of plate glass, permits direct observation and the use of a reading glass 4 inches in diameter. The temperature in the incubator was permitted to vary from 80° to 100°F. The higher temperatures were accompanied by an unusual amount of activity. The young of the 7th day may live as long at room temperature (about 75°F) as in the incubator, but they are not nearly so active. One specimen of the 7th day was kept in the incubator from 9 till 10:30 A. M. at a temperature of 82° to 85° and then removed and placed in a glass dish on a table where it lived till 10:50 P. M., but it was constantly pale and cold to the touch, and very inactive, whereas a litter mate kept in the incubator was dead in 12 hours and 20 minutes, and another was living after 11 hours and 38 minutes. In the incubator the young were placed on pieces of outing flannel saturated with mineral oil to prevent desiccation of the skin.

During the first day in the pouch the young hold the feet closely appressed to the tail, which is bent forward closely on the ventral surface of the body. Of the hind leg only the foot extends beyond the contour of the body. The toes and claws are sufficiently strong so that one can hook a needle under them and drag the animal around. Spontaneous (endogenous) movements are almost constant when the young are kept warm. There is a great deal of individual movement of the fore limbs. The movements of the trunk begin in the head or neck region and progress caudad as an integrated system which effects alternate movements of the arms, with the head extended and moving from side to side. With ventral flexion

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* This work was aided by a grant from the Josiah Macy, Jr., Foundation.

(indicating a functional m. rectus abdominis) and extension, and squirming movements of the trunk, the hind legs do not move. No movement can be seen in them except that with a lens a very slight abduction is rarely perceptible when the body undergoes extreme ventro-flexion. This is probably due to lateral pressure of the viscera in the pelvis. The movements of the fore limbs may be characterized as grasping movements. They consist of extension of the fore limb, hand and fingers to full length and flexion of the fingers in initial phase of flexion of the limb. The result is a grasping of any suitable object, as the fur of the mother or the fibers of a rough cloth, with which they come in contact. The young take advantage of this and raise themselves to an upright position supported by the snout, rump and forelimbs, and crawl about by means of alternate movement of the fore limbs, extension of the head and pulling with the snout, aided by the anchorage provided by the claws of the hind feet. In this erect position they may lift the head high in the air and move it from side to side, while the claws cling to the cloth. Specimens placed between pieces of flannel crawled out and were found lying on the piece that had covered them. With lowered vitality as the young approached death there was a great deal of individual movement of the fore limbs.

In the 4th day the hind feet are held in the same position as in the first and do not move otherwise than in the first day. There are small bilateral movements of the arms. There is much ventroflexion of the rump only, indicating the action of the m. rectus abdominis alone. One specimen rolled and squirmed for 5 minutes, with very little if any progress, then definitely assumed the erect position and crawled its full length in 4½ minutes. After this it lay still, and I lifted the edge of the cloth on which it lay, progressively through various angles till it was in a horizontal position with the young suspended beneath by the claws of the fore and hind feet. I manipulated the cloth in various planes, and the young held fast. On the part of the hind feet this clinging must be passive inasmuch as the hind limbs do not move at this time. Afterward it crawled the distance of 2 cm. When I held the young in my fingers the head ventroflexed and the fore feet were brought up to the chin.

In a specimen that, judged by measurement, was slightly older there was still no movement of the hind legs. There were extensive grasping movements of the fore limbs at full length and abducted. In the typical resting position the hands are folded over the hind legs and the chin appressed to the chest or to the hind feet. In their movement the fore legs manipulate the hind legs a great deal.

The young of the seventh day still hold the feet closely appressed to the tail, which extends 1-2 mm in front of the feet. There is still a slight abduction of the hind legs on extreme ventro-flexion of the trunk, due presumably to pressure in the pelvis, and in addition there is very rarely an extension and flexion of the hind legs with movement of the trunk, but no movement in them without movement of the trunk. The 7 specimens of this age were under observation for a total of over 67 hours, during which time they were examined 35 times critically for movement of the legs with movement of the trunk. Of these trials 19 were negative, 4 were questionable and 12 were positive. These movements were so slight and rare that the 7th day may be regarded as marking the beginning of movement of the hind legs as a part of a total pattern of action.

In a litter of 6 young (measuring 22 mm c. r. length in 20% formalin) there was movement in knee and ankle joints with movement of the trunk, but no movement in the joints of the foot, and there was no movement of the hind leg unless the trunk and fore legs moved. When both fore legs moved both hind legs moved. When I took the head in one hand and the tail in the other and stretched the specimen out ventral side up there was rotation of the body from side to side and alternate movement of the fore legs with the hind legs moving mostly in harmony with the fore legs—when the fore leg on one side went forward the hind leg of the same side went backward—essentially the walking gait. The hind legs, however, do not always move alternately at this age, nor in harmony with the fore legs.

In the young of the first day a touch of a bristle on the snout may be followed by ventro-flexion of the trunk and flexion of the fore legs, or it may cause ventro-flexion of the rump only. Raking the bristle across the snout causes a slight ventro-flexion of the neck and trunk, extending to the rump. This response was repeated 8 times. Drawing the bristle down over the rump was followed by slight ventro-flexion of the rump only; sometimes the head moved in this response.

On the 4th day sometimes stimulation on the rump caused movement of the head and fore legs and sometimes ventro-flexion of the rump only. In a slightly older specimen (as judged by measurement) the prick of a needle in the rump caused first movement of the rump that continued farther headward and into the fore limbs, followed by about 3 minutes of general activity. At another time the same stimulus caused ventro-flexion of the rump, then movement of the fore limbs and head. Sometimes, however, a stab of the needle in the rump produces only slight ventro-flexion of the rump.

A prick of the needle on the forearm caused movement of the same fore limb and then other parts, or a touch on one fore limb caused extension of both fore limbs. Stimulation of the left fore foot with a brush caused flexion in both fore feet.

In specimens of 22 mm c. r. length (in 20% formalin) a prick of a needle on the rump was followed by a general movement, but the hind legs did not move independently. Repeated pricks of the needle in the hind foot and shank as far up as the knee gave no responses. I held the young in my fingers and spread the foot out on my thumb nail, and pricked the foot in several places, the ankle and shank but there was no response. This test was repeated with the same result. I then amputated the foot with iridectomy scissors and there was no response. In other cases I split the foot longitudinally as far up as the ankle joint, thinking it would have more freedom to move than with amputation, but there was no response.

Hartman[1] reported that he removed embryos of the opossum from the uterus at near term and that they crawled over the hair of the mother. Weed and Langworthy[2] placed the pouch-young on gauze, although the youngest animal they reported on was 23 days old with a length of 33 mm, and it grasped the cloth with the claws of the fore legs. Langworthy[3] reported that, when young of 10 days (22 mm) were placed on gauze, they "would seize the threads with the claws of the fore legs" and pull themselves along. In a later paper[4] he says of opossums of 7 days (27 mm), when placed on gauze, "the claws of the fore legs seized the threads and the young pulled itself along by alternate progressive beats of the fore legs," and "the hind legs did not participate in these grasping movements, but remained for the most part in a flexed position." He found there was no righting reflex in specimens of 36 days, but that it was beginning to develop at 41 days, and is "not yet very active" at 46 days.

My opossums of the first day in the pouch rolled over from one side to the other ventral side down, and took the erect position by clinging to the flannel with the fore legs, and crawled in that position. This was purely thigmotactic, for we know from the observations of Larsell, McCrady, Jr., and Zimmerman[5] that the vestibular apparatus is not functional at that time. This thigmotactic righting reaction may be a transitory function characterizing the period from immediately before till a few days after birth, serving the animals, under normal conditions, only for gaining access to the pouch and the nipple (Hartman[1]). I did not observe it in specimens of 28 mm c. r. length, but I did not examine them critically for this feature.

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[1] Hartman, C. G., *Anat. Record*, 1920, **19**, 251.

[2] Weed, L. H., and Langworthy, O. R., *Am. J. Physiol.*, 1925, **72**, 8.

[3] Langworthy, O. R., *Contributions of Embryology, Carnegie Inst. Wash.*, 1927, **19**, 149.

[4] Langworthy, O. R., *J. Comp. Neurol.*, 1928, **46**, 201.

[5] Larsell, Olof, McCrady, Edward, Jr., and Zimmerman, Arnold Albert, *J. Comp. Neur.*, 1935, **63**, 95.

With regard to coördination of the hind legs with the fore legs, Langworthy[4] says that there was very little contraction of hind leg musculature "at an estimated age of 7 days" (27 mm), and scarcely any coördination "at 36 days" (49 mm). Possibly his failure to find it earlier may be due to an interference of local fields of stimulation with the mechanism of the total pattern action (Coghill[6]). The position in which I held the animal might be favorable for the free action of the limbs.

Conclusions. From my observations it is clear that, in the opossum of the first day in the pouch, there are functional commissural relations at the level of the brachial plexus, a descending (motor) path as far caudad as the lower thoracic level (for stimulation of the m. rectus abdominis) and an ascending pathway from the lumbosacral region to the upper cervical segments or the brain; also that the hind legs become motile on the 7th day in the pouch, and at a more advanced stage they become integrated with the fore legs so as to execute the walking gait before they are capable of reflex action. In the development of walking, therefore, in the opossum as in amblystoma (Coghill[7]), the gait is essentially a total pattern of action while reflexes serve for orientation and adjustment to surfaces.

10082

Excretion of Sex Hormones in Urine of Adult Male Monkeys.*

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The concentration of androgenic activity found in the urine of human males has been shown to be high in comparison to that found

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[6] Coghill, G. E., *Psychiatrische en Menrologische Bladen*, 1934, No. 3 and 4, 122.

[7] Coghill, G. E., Proc. Soc. Exp. Biol. and Med., 1929, **27**, 74.

* Aided by a grant from the General Education Board of the Rockefeller Foundation and by a grant from the Research Fund of the Yale University School of Medicine.

in the urine of stallions, bulls and rams.[1] It becomes of interest, therefore, to study the quantitative excretion of androgenic substances in the urine of the adult male monkey.

TABLE I.
Excretion of Androgenic and Estrogenic Substances in Urine of Adult Male Monkeys.

Monkey	Body weight kg	No. of days' sample	I.U.* per 24 hr Estrogenic activity	I.U.* per 24 hr Androgenic activity
A	9.0	4	2.1	1.7
A		3	—	2.4
A		4	2.0	1.3
A		4	2.5	4.1
B	10.0	4	2.1	3.8
B		3	—	1.4
B		4	1.3	4.7
B		4	<2	1.9
C	6.9	4	—	1.0
C		4	<2	3.2
C		4	—	1.9
C		4	1.1	3.7

* The International Unit of androgenic activity is that evoked by 0.1 mg of androsterone. The I.U. of estrogenic activity is that evoked by 0.1 mg of estrone.

Three normal adult rhesus monkeys of known fertility weighing between 6.9 and 10.0 kg were chosen for the urine collections. Four samples from each monkey were collected over periods of 72 or 96 hours. The method employed for the quantitative extraction of androgenic and estrogenic substances from the urines has been previously described.[2] The androgenic activity was assayed by means of the day-old chick's comb,[3] while the estrogenic activity was determined by means of the estrus reaction in the adult spayed female mouse.

The results of the assays of both estrogenic and androgenic activity are listed in Table I. It is shown that the adult male monkey excretes both estrogenic and androgenic substances. The estrogenic activity varied from 1.1 to 2.5 I.U. per 24 hours, while the androgenic activity varied from 1.0 to 4.7 I.U. per 24 hours.

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[1] Koch, F. C., *Physiol. Rev.*, 1937, **17**, 165.

[2] Gallagher, T. F., *et al.*, *J. Clin. Inv.*, 1937, **16**, 695.

[3] Dorfman, R. I., and Greulich, W. W., *Yale J. Biol. and Med.*, 1937, **10**, 79.

10083

Failure of Sulfanilamide to Inactivate Preformed Hemotoxins, Diphtheric Toxin, or Tetanal Toxin.

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Experiments on the action of sulfanilamide on *beta* hemolytic-streptococcal infections in cultures of human marrow[1] indicated that sulfanilamide, in some manner, interfered with the production of the toxins responsible for the virulence of the organism and for its resistance to small amounts of bactericidins. To determine whether sulfanilamide would inactivate preformed toxins or affect other toxins, the experiments herein described were performed.

Beta hemolytic streptococci, strain AB-13, were grown in Hartley broth for 16 to 24 hours and the Seitz filtrate was tested for hemotoxin with rabbit, human, and sheep cells. No hemotoxin was demonstrable even when the filtration was carried out in an atmosphere of nitrogen and reducing agents were added to the filtrate. Therefore, unfiltered 16- to 24-hour cultures were diluted with saline or saline plus sulfanilamide, and incubated for one hour in a 37.5° waterbath, with equal volumes of a 1% saline suspension of rabbit, human, or sheep cells. The concentrations of sulfanilamide used were 1-1000, 1-10,000, and 1-100,000. When this technic was employed, complete hemolysis occurred in all tubes containing sulfanilamide corresponding in dilution to the controls which showed complete hemolysis. However, hemolysis became complete from 1 to 15 minutes earlier in the controls than in the corresponding dilutions containing sulfanilamide. Since this might be explained by bacteriostasis, similar experiments were performed in which 0.1 volume of 1-1000 aqueous merthiolate was added to each tube to prevent further bacterial growth. With this precaution no differences were noted between the tubes containing sulfanilamide and the corresponding controls. If the hemolytic streptococci were inoculated in Hartley broth, the cultures divided into 2 equal parts and sulfanilamide added to one, the production of hemotoxin was delayed but not prevented in the culture containing sulfanilamide.

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[1] Osgood, E. E., *J. A. M. A.*, 1938, **110**, 349; Osgood, E. E., and Brownlee, Inez E., *J. A. M. A.*, 1938, **110**, 1770.

Hemotoxin* from the hemolytic *Staphylococcus aureus* was not inactivated by sulfanilamide in concentrations up to 1-1000, nor was a sample of *B. perfringens* hemotoxin.†

Hemotoxins were prepared as filtrates of *Cl. tetani* (48-hour), *Cl. oedematis-maligni* (16-hour), and *B. perfringens* (16-hour) broth cultures, and as filtrates of hemolytic *Staphylococcus aureus* (48-hour) 0.2% semisolid agar cultures.‡ Incubation at 32°C seemed to favor especially the production of tetanal hemotoxin. The minimal hemolytic doses varied from 1/8 to 1/64 cc for an amount of washed cells equivalent to 1/40 cc of whole blood. Human, sheep, and rabbit erythrocytes were not hemolyzed to the same extent. The data herewith given relate to human cells.

Attempts were made to inactivate the various hemotoxins with sulfanilamide. To each tube of a series of progressively halved concentrations of sulfanilamide, 1-500 to 1-8,192,000, were added one minimal hemolytic dose of hemotoxin, and one test-dose of erythrocytes; final volume, 1.0 cc. Incubation was conducted for one hour at 37°C, proper control tubes being included.

No inactivation of the hemotoxins of *Cl. tetani, Cl. oedematis-maligni,* or hemolytic *Staphylococcus aureus* was demonstrable by the above method. A variation in method comprising 30 minutes' contact of hemotoxin and sulfanilamide in the icebox, followed by the addition of erythrocytes and regular incubation caused no inactivation of these 3 hemotoxins. On the other hand, sulfanilamide gave what appeared to be partial inactivation of the *B. perfringens* hemotoxin; the degree of hemolysis being 2 plus with all concentrations of sulfanilamide down to and including 1-4,096,000. However, with additional incubation of an hour or two at room temperature, hemolysis became complete. The reason for this slowing of hemolysis, simulating inactivation of one of two or more hemotoxins by antitoxin, has not yet been determined, although it was noted in hemotoxin aged up to 10 days but not 15 days or over.

Experiments were conducted in which various concentrations of sulfanilamide were incorporated in *B. perfringens* culture-broths in which hemotoxin was to be produced. It was found that concentrations of sulfanilamide of 1-10,000, 1-20,000, and 1-50,000 did not interfere with the production of hemotoxin or growth of the culture as compared to control cultures without sulfanilamide. All 4 of these samples of hemotoxin had an MHD of 1/64 cc.

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* We are indebted to Doctor H. J. Sears of the Department of Bacteriology, University of Oregon Medical School, for the preparation of this hemotoxin from strain Dolman Wood 46.

† Supplied by Eli Lilly and Company, Indianapolis, Indiana.

‡ All of the experiments on hemotoxins were performed in the Biological Research Division, Eli Lilly and Company, Indianapolis, Ind.

Saponin in a dose of 0.5 cc of a 1-4000 dilution was fully hemolytic for our test-dose of erythrocytes. This hemotoxin was observed, as is well known, to be enhanced in potency by benzol, the increase being 8-fold when 1-10,000 benzol was used. Sulfanilamide, however, did not affect the hemolytic action of saponin.

We have attempted without success to cure guinea pigs treated with diphtheric or tetanal toxins. With these toxins, *in vivo* tests

TABLE I.
Effects of Sulfanilamide on Survival of Guinea Pigs Given Diphtheric Toxin.

Controls			Sulfanilamide-Therapy			
No. of pigs	MLD	Hr survival	No. of pigs	MLD	Dose of sulfanilamide	Hr survival
					mg	
1	100	17	1	100	2 initial	17
1	50	17	1	50	2 ''	17
1	25	17	1	25	2 ''	17
2*	10	<96	2*	10	50 at 1 hr 50 '' 5 ''	<96
2*	5	<96	2*	5	50 '' 1 '' 50 '' 5 ''	<96
2	2.5	21.5, 22	2	2.5	2 initial 2 q. 4 h.	21.5, 22
2	1.25	19.5, 84	2	1.25	2 initial 5 q. 4 h.	41.5, 72
4*	1	<96	4*	1	50 at 1 hr 50 '' 5 ''	<96

* Performed in Indianapolis.

TABLE II.
Effects of Sulfanilamide on Survival of Guinea Pigs Given Tetanal Toxin.

Controls			Sulfanilamide-Therapy			
No. of pigs	MLD	Hr survival	No. of pigs	MLD	Dose of sulfanilamide	Hr survival
					mg	
5	105	25, 28, 30, 32, 32	5	105	5 q. 4 h.	33, 34, 34, 41.5, 44
3	60	28, 31, 40	3	60	5 q. 4 h.	38, 45, 45
12	30	2 lived, 36, 36, 36, 40, 40, 43, 48, 50, 50, 53	12	30	5 q. 4 h.	5 lived, 35, 35, 41, 46, 48, 50, 50
1	12	lived	1	12	2 q. 4 h.	lived
2*	10	<96	2*	10	75 at 1 hr 75 '' 5 ''	<96
2*	5	<96	2*	5	75 '' 1 '' 75 '' 5 ''	<96
1	3.5	lived	1	3.5	2 q. 4 h.	lived
1	1.5	''	1	1.5	2 q. 4 h.	''
4*	1	<96	4*	1	75 at 1 hr 75 '' 5 ''	<96

* Performed in Indianapolis.

obviously could yield more important results than could *in vitro* tests. It may be mentioned that diphtheric toxin is devoid of hemotoxin, while tetanal toxin as usually produced (by incubation of the culture for about 2 weeks) is also devoid of hemotoxin but contains considerable hemotoxoid.

It will be noted (Tables I and II) that sulfanilamide-therapy had absolutely no effect on the survival of guinea pigs receiving diphtheric toxin, and very little effect on those receiving tetanal toxin. It seems probable that in the experiments with tetanal toxin the slight differences in favor of the guinea pigs receiving sulfanilamide may be explained by chance variation.

Conclusion. Sulfanilamide in concentrations of 1-1000 or less does not inactivate *in vitro* significant amounts of the hemotoxins of the *beta* hemolytic *streptococcus,* hemolytic *staphylococcus aureus, Cl.oedematis-maligni, Cl. tetani,* or *B. perfringens.* Sulfanilamide-therapy did not significantly affect the course of intoxication in guinea pigs initiated with as little as 1 MLD of diphtheric or tetanal toxin.

10084

Preparation of Extracts of the Renal Pressor Substance.*

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During recent years, evidence has accumulated which suggests that elevation of blood pressure may be produced by some substance or substances which occur in renal tissue. In the course of investigations on the relationship of certain types of experimental hypertension to this renal pressor substance, one difficulty frequently encountered has been that of obtaining uniform and potent renal extracts. An attempt was made to determine the factors which affect the yield of this pressor substance, and to develop a standardized method of preparation.

Fresh kidneys of hogs were obtained from the slaughter house. The kidney cortex was dissected with scissors from the medulla, and the cortical tissue passed through a meat grinder. The minced kidney cortex was ground to a fine paste with carborundum. The possibility that carborundum might adsorb some of the pressor substance was

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* Aided by a grant from the Josiah Macy, Jr., Foundation.

excluded by comparing yields from fractions ground with large amounts, small amounts, and none of this substance. Comparisons of fractions ground for different periods of time were also made. The observations showed that a better yield was obtained when the tissue was macerated in a mortar with carborundum, and that the amount of abrasive and the duration of grinding did not influence the yield appreciably, provided that the tissue was ground to a uniform paste.

Ninety-five percent alcohol was added to the paste in an amount equal to 9 cc per gram of original kidney cortex. The time required to obtain maximum precipitation of the pressor substance was investigated. It was found that when the alcohol was separated within a few minutes, the residue was less active than that obtained from a kidney-alcohol mixture which had been allowed to stand in the ice-box for 24 hours. Longer periods of standing—up to 10 days—decreased the activity of the final product. The temperature at which the mixture was kept was found to be of importance; residues from mixtures kept in an incubator at 37°C were less active than

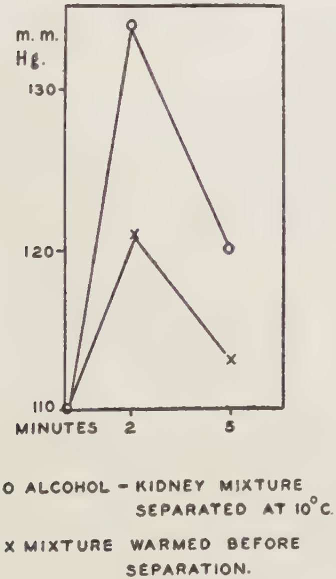

FIG. 1.

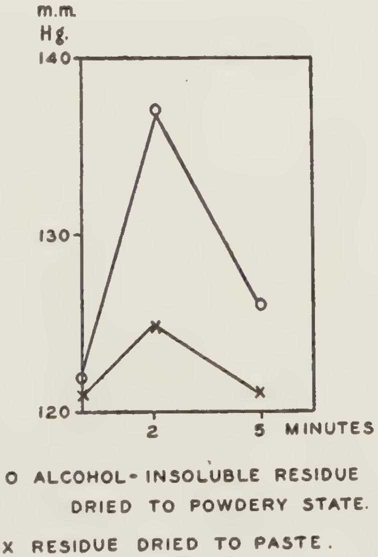

FIG. 2.

Effect of temperature on potency of extract (average of 8 comparisons).
o: Alcohol-kidney mixture separated at 10° C.
x: Mixture warmed before separation.

FIG. 2.

Effect of drying on potency of extract (average of 7 comparisons).
o: Alcohol-insoluble residue dried to powdery state.
x: Residue dried to paste.

those from similar material kept in the refrigerator at approximately 10°C. Centrifuging the mixture as soon as it was taken from the refrigerator, and as rapidly as possible, resulted in preparations which were somewhat more active than those obtained from material which was allowed to stand at room temperature before separation. (Fig. 1.)

After the alcohol-soluble fraction had been decanted and discarded, the alcohol-insoluble residue was washed once with 2 to 4 volumes of ether. The mixture was centrifuged again, the ether discarded, and the sediment spread out on flat plates and dried in a current of air at room temperature. Material desiccated to a powdery state yielded more potent extracts than material which had been dried only to a paste which was free of alcohol and ether. (Fig. 2.) The ether washing resulted in more rapid drying and removal of a lipoidal cloud from the final preparation, without causing any diminution in potency.

The dried powder was triturated with various aqueous solutions. It was found that somewhat better yields of pressor substances were obtained when 0.5% sodium bicarbonate was used than when water or slightly acid sodutions were employed, and that the extract was most potent when the sodium bicarbonate solution was warmed to 40 to 45°C. The amount of solution added to the dry powder was equal to 1 cc per gram of original cortical tissue. After centrifuging, the residue was twice reëxtracted with one-half this volume of warm sodium bicarbonate solution, and the 3 fractions of supernatant fluid obtained by the serial extraction combined.

Attempts were made to further purify the preparations by reprecipitation of the alcohol-insoluble pressor substance. Nine volumes of alcohol were added to one volume of the aqueous extract. The precipitate which formed in this mixture, after it had stood in the refrigerator for 24 hours, was then separated and treated as described above. "Refined" extracts of this type did not differ in their physical and pharmacological characteristics from the original extracts. It was found, however, that quantitative recovery of the pressor substance could not be even approximated (Fig. 3), and this attempt at refinement of the preparation was therefore abandoned. Attempts at isolation of the active pressor principle by salting out with various concentrations of ammonium sulphate, and subsequent dialysis, were also unsuccessful.

The final extract, prepared as described above, is a slightly turbid solution, which has a pronounced and prolonged pressor effect when injected into the experimental animal. The white rat has been found

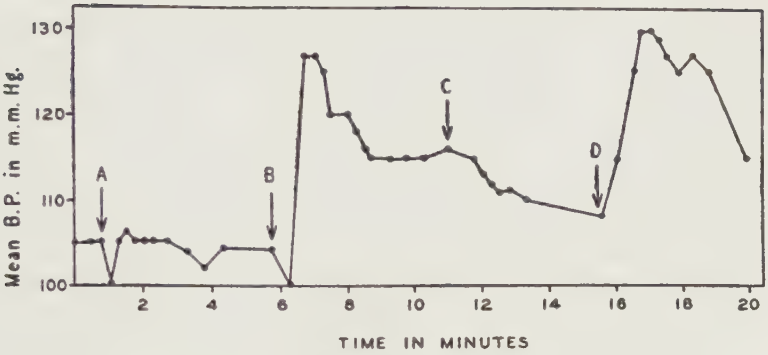

Fig. 3.

Loss of potency of extract on reprecipitation. Heat inactivation of renin.

A: Injection of extract from 0.25 g kidney, "refined" by 2 precipitations from alcohol, then heated at 60°C for 10 minutes.

B: Same as A, but extract not heated.

C: Extract from 0.12 g kidney, not reprecipitated, heated at 60°C for 10 minutes.

D: Same as C, but not heated.

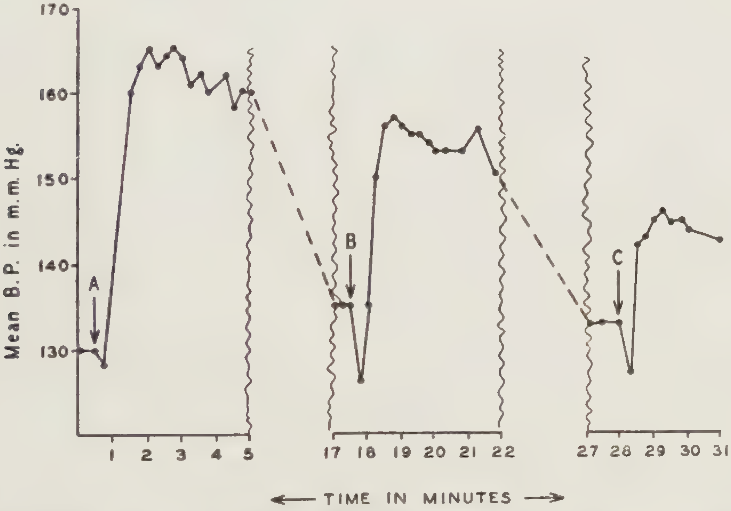

Fig. 4.

Diminishing effect of successive injections of renin. Influence of dialysis on potency of extracts. At points A and C, dialyzed extracts representing 0.25 g kidney injected; at B, equivalent amount of extract which had not been dialyzed.

to be a very satisfactory test animal. Repeated injections into the same animal result in progressively diminishing responses. (Fig. 4.) The pressor substance is heat labile, and is destroyed by exposure to a temperature of 60°C for 10 minutes. (Fig. 3.) It is not dialyzable through Visking membranes. (Fig. 4.) Its effect is not abolished by cocainization of the test animal. Application of the same extractive technic to other organs (spleen, liver) does not yield a preparation of similar pharmacological properties.

Summary. Some technical factors which are of importance in the preparation of extracts of the renal pressor substance (the "renin" of Tigerstedt and Bergmann) have been investigated. A method which has been found to be satisfactory for the preparation of potent extracts of this substance is described in detail.

10085 P

Metabolism of Nitrogen, Calcium, Magnesium and Phosphorus in Thymectomized Rats.

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In the course of our work on the influence of the glands of internal secretion on mineral metabolism in albino rats, nitrogen, calcium, magnesium and phosphorus metabolism in thymectomized rats was studied.

Twenty male and 22 female albino rats were used, 6 animals of each sex being kept as paired feeding controls. They were thymectomized at 7 weeks of age. Details regarding procedure and meth-

TABLE I.

	Nitrogen Metabolism in							
	Thymectomized Rats				Paired Feeding Controls			
	% of Intake				% of Intake			
Date	Total N Urine	Excretion Feces	Intake mg	% of Intake Retent.	Total N Urine	Excretion Feces	Intake mg	% of Intake Retent.
Control Period:								
Feb. 25-Mar. 2	11	10	294	79	15	10	290	75
Mar. 3-9	15	12	265	73	17	11	268	71
*								
Mar. 14-18	28	21	291	51	23	18	290	59
Mar. 19-23	25	24	287	51	18	15	290	67
Mar. 24-30	29	20	297	51	18	19	302	63
Mar. 31-Apr. 6	27	19	297	54	21	20	300	59
Apr. 7-13	33	24	320	43	20	19	318	61
Apr. 14-20	39	23	313	38	22	20	315	58
Apr. 21-27	37	23	310	40	18	19	308	63
Apr. 28-May 4	38	20	310	42	25	21	311	54
May 5-11	38	21	310	41	24	17	313	59
May 12-18	40	22	342	38	25	16	340	59

*Thymectomized on March 11th.

ods of analysis will be found in a previous publication.[1] Urinary nitrogen excretion increased progressively from 13% of the intake during the control period to 40% 10 weeks after operation. In the paired feeding controls the urinary N excretion increased from 16% of the intake to 25% only, during the same time. The increase in N excretion was accounted for by urea N. Fecal N excretion, expressed in percentage of intake, remained unchanged. The drop in N retention was more pronounced in the thymectomized rats than in the paired feeding controls, though a positive N balance was maintained at all times and the gain in weight was normal in both groups. No difference was found between thymectomized and control animals regarding the excretion of NH_3 nitrogen, uric acid, creatinine and creatine. There was no change in the calcium, magnesium and phosphorus metabolism.

Our studies are being extended to include the metabolism of sulfur and glutathione in thymectomized rats.

10086

Immunizing Effect of Old Tuberculin on Experimental Tuberculous Infection.

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Several years ago Rothschild, Friedenwald, and Bernstein[1] showed that hypersensitivity could be separated from immunity in experimental tuberculosis in the guinea pig. After rendering the animals both hypersensitive and immune by infection with an attenuated strain of tubercle bacilli, they desensitized them with large doses of Old Tuberculin. Upon reinfection with virulent organisms, the animals' immunity was found to be unimpaired. These observations have been confirmed by other investigators.[2]

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[1] Sandberg, M., and Perla, D., *J. Exp. Med.*, 1934, **60**, 395.

[1] Rothschild, H., Friedenwald, J. S., and Bernstein, C., *Trans. Nat. Tuberc. Assn.*, 1931, p. 149; *Bull. Johns Hopkins Hosp.*, 1934, **54**, 232.

[2] Boquet, A., *Compt. Rend. Soc. Biol.*, 1932, **112**, 1168; Cummings, D. E., and Delahant, A. B., *Trans. Nat. Tuberc. Assn.*, 1934, p. 123; Derick, C. L., Branch, E. A. G., and Crane, M. P., *Am. Rev. Tuberc.*, 1934, **32**, 218; Siegl, J., *Beit. z. klin. d. Tuberk.*, 1934, **84**, 311; Birkhaug, K., *Acta Tuberc. Scand.*, 1937, **11**, 25; Higginbotham, M. W., *Am. J. Hyg.*, 1937, **26**, 197.

In the experiments of Rothschild, *et al.,*[1] extremely large amounts of Old Tuberculin were administered, a total of 142 cc per animal over a period of 126 days. This to our knowledge is more than had ever been given before. Recently Topley and Wilson[3] have raised an objection to "the assumption that the only effect produced was the desensitization of the animals. The possible immunizing effect of the tuberculin itself is altogether ignored."

To determine whether the administration of large amounts of Koch's Old Tuberculin might have some immunizing effect when given both before and during the course of experimental tuberculous infection, the present study was undertaken.

Tuberculin was made by growing virulent organisms for 8 weeks on Proskauer and Beck's[4] synthetic medium. The bacilli were removed by filtration through paper and the filtrate passed through a Berkefeld N candle. After evaporation to one-tenth its original volume, the tuberculin was stored in the ice-chest. Synthetic medium of the same composition was filtered and concentrated in exactly the same manner to make the control concentrated medium. The tubercle bacilli used both for infection and in making the tuberculin were from a virulent strain which was isolated several years ago from the sputum of a human case of active pulmonary tuberculosis. It has been grown continuously on Proskauer and Beck's medium at a pH of 7.4 with no appreciable change in its virulence. A 3-weeks-old culture was used for inoculation.

Thirty adult male guinea pigs, weighing about 500 g, were divided into 3 groups. Ten received 2 cc undiluted Old Tuberculin subcutaneously every other day for 6 weeks (42 cc). Ten more received the same amount of concentrated medium (42 cc) every other day, while the remaining 10 received nothing. At the end of the 6-weeks interval all the animals were tested intracutaneously with 50 mg Old Tuberculin and all gave no reaction. All were then infected in the left groin with a suspension of virulent human tubercle bacilli containing 5-6 organisms per oil immersion field. Treatment with both Old Tuberculin and concentrated medium was continued with daily injections of 2 cc of each for 44 more days when all were killed with ether.

Two days before death all animals were tested intracutaneously with 0.1 cc of a suspension of whole live virulent tubercle bacilli. Those treated with tuberculin gave negative reactions while the 2 control groups gave markedly positive Koch phenomena with necrosis. These skin tests were made on the left side, the entire right side being reserved for the daily injections of tuberculin or concentrated medium. At autopsy the tissues were fixed in Zenker-formol solution. Sections of lungs, lymph nodes, livers and spleens for microscopical study were stained with hematoxylin and eosin. Sections of lung and spleen were stained with carbol fuchsin.

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[3] Topley, W. W. C., and Wilson, G. S., "The Principles of Bacteriology and Immunity," 2nd Ed., 1936.

[4] Baldwin, E. R., Petroff, S. A., and Gardiner, L. S., "Tuberculosis. Bacteriology, Pathology, and Laboratory Diagnosis," 1927.

Of the original 30 animals, 18 survived the treatment 23 or more days after infection. One from each group came to autopsy on the 23rd day while all the remaining 15 animals (5 from each group) were killed on the 44th day after infection.

There was very little difference in the extent of the lesions in the 3 groups. As is usually the case in experimental tuberculosis in guinea pigs, the spleens were most extensively involved, then the livers, and lastly the lungs. The difference which was found was one of character. When the lungs and spleens were carefully examined there was found to be a distinct tendency for the tuberculous foci in the Old Tuberculin treated animals to show less caseation than the controls although the former group was not entirely free from caseous lesions.

As far as the number of bacilli in the lungs and spleens of the 3 groups was concerned, all contained comparable numbers with one exception. This was No. 71 of the Old Tuberculin treated group. In the pulmonary alveoli of this animal there were enormous numbers of acid-fast bacilli apparently growing unchecked. There were also numerous polymorphonuclear as well as mononuclear leucocytes but the striking thing was the great proliferation of acid-fast organisms. This type of lesion is exactly similar to that which has recently been described by Willis and Woodruff[5] and which we have seen in other experiments.[6] The former investigators have suggested that this lesion develops as a result of lack of hypersensitivity. We have taken up this question in another place[6] where it is pointed out that this lesion may also develop in hypersensitive animals.

In experimental tuberculosis practically all investigators agree that a definite immunity is conferred by vaccination with living attenuated bacilli or heat-killed virulent organisms. To our knowledge no one has succeeded in producing any demonstrable immunity with fractions of the tubercle bacillus, or with the products of its break-down in cultures, *e. g.,* Old Tuberculin. However, because only recently has it been realized that animals will tolerate large daily doses of Old Tuberculin, it seemed possible that some degree of immunity might be conferred by treatment with large amounts of this material previous to and during infection.

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[5] Willis, H. S., and Woodruff, E. C., *J. Clin. Invest.,* 1937, **16**, 899; *Am. J. Path.,* 1938, **14**, 337.

[6] Follis, R. H., Jr., in press.

In 1910 Krause[7] had given guinea pigs as much as 25 cc of water extract tuberculin before infection, with no apparent beneficial effects. A little later Klopstock[8] administered 24 cc of Old Tuberculin over a period of 149 days before infection, and found no significant decrease in tuberculosis between treated guinea pigs and their controls. In the present experiment a total of 130 cc of undiluted Old Tuberculin were administered, 42 cc before infection and 88 cc over the 6-weeks period following inoculation with virulent bacilli.

From the results of our experiment, therefore, it seems quite unlikely that Old Tuberculin contains any substance which plays a rôle in augmenting the guinea pig's power to prevent the spread and hinder the growth of the tubercle bacillus or to destroy this organism. And it is thus apparent that in the experiments of Rothschild, *et al.*,[1] as well as those of other observers, the administration of large doses of Old Tuberculin for the purpose of desensitization, did not play a rôle in bringing about the resistance to infection which animals deprived of their hypersensitivity exhibit. Therefore, we do not feel that the objection raised by Topley and Wilson[3] is a valid one. The only effect of continued large doses of Old Tuberculin was on the character of the lesions; those in the treated animals showed less caseation than those in the controls. In the light of the general knowledge concerning the action of hypersensitivity in rendering tissues susceptible to injury by bacterial products, the fact that desensitization decreased the amount of necrosis in these experiments and those of others, is not surprising.

Summary. Old Tuberculin when administered subcutaneously in large amount both before and during experimental tuberculous infection in guinea pigs, confers no demonstrable immunity. The view that the immunity that persists after desensitization by tuberculin is a result of the treatment is, therefore, not a valid one.

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[7] Krause, A. K., *Studies from the Saranac Laboratory for the Study of Tuberculosis*, 1911, p. 1.

[8] Klopstock, F., *Z. f. exp. Path. u. Ther.*, 1913, **13**, 56.

10087 P

Tuberculous Infection in Animals Prevented from Developing Hypersensitivity.

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Several investigators[1] have shown that guinea pigs rendered hypersensitive and immune with attenuated tubercle bacilli can be desensitized with Koch's Old Tuberculin (O.T.) without loss of their immunity. The present study was begun with the object of investigating the effect of preventing the development of hypersensitivity in experimental tuberculous infection. Since beginning these experiments Willis and Woodruff[2] have reported investigations of like nature, with the conclusion that preventing the development of hypersensitivity made animals unusually susceptible to tuberculosis.

Our first series consisted of 16 adult male guinea pigs. Half were given 1 cc undiluted O.T. subcutaneously daily for 10 days and 2 cc daily thereafter until death. The remaining 8 received nothing and served as controls. On the 4th day all were inoculated in the groin with 0.1 cc of a suspension of a virulent strain of human type tubercle bacilli (5-6 organisms per oil immersion field). Intracutaneous tests were made with 20 mg O.T. at 20 and 37 days and with a suspension of living bacilli 40 days after infection. All treated animals gave negative reactions while the controls gave positive reactions to the tuberculin and characteristic Koch phenomena to the bacilli. At autopsy no differences were found grossly or microscopically in 5 treated animals and their controls dying in the 4th week. Little caseation was found in either group. However, in the 3 remaining treated animals living to the 42nd day, there appeared lesions of an extraordinary nature. In the lungs, many alveoli were packed with polymorphonuclear and mononuclear leucocytes together with enormous numbers of acid-fast bacilli. The liver and spleen of these animals revealed relatively few bacilli in comparison. No such lesions were found in the controls. These changes are similar to those described by Willis and Woodruff.[2]

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[1] Rothschild, H., Friedenwald, J. S., and Bernstein, C., *Trans. Nat. Tuberc. Assn.*, 1931, p. 149; *Bull. Johns Hopkins Hosp.*, 1934, **54**, 232; Boquet, A., *Compt. Rend. Soc. Biol.*, 1932, **112**, 1168; Cummings, D. E., and Delahant, A. B., *Trans. Nat. Tuberc. Assn.*, 1934, p. 123; Derick, C. L., Branch, E. A. G., and Crane, M. P., *Am. Rev. Tuberc.*, 1934, **32**, 218; Siegl, J., *Beit. z. klin. d. Tuberk.*, 1934, **84**, 311; Birkhaug, K., *Acta Tuberc. Scand.*, 1937, **11**, 25; Higginbotham, M. W., *Am. J. Hyg.*, 1937, **26**, 197.

[2] Willis, H. S., and Woodruff, C. E., *J. Clin. Invest.*, 1937, **16**, 899; *Am. J. Path.*, 1938, **14**, 337.

Series II consisted of 61 animals living 36 to 80 days. Eighteen were treated with daily subcutaneous injections of O.T. from the day of infection on, half receiving 1 cc and the rest 2 cc each day. Twenty-two were treated with injections of 10% glycerin in saline (since O.T. contains glycerin), 10 receiving 1 cc and twelve 2 cc each day. Twenty-one received daily injections of 0.9% saline, 11 receiving 1 cc and ten 2 cc. The infecting dose in this group was a suspension containing about 20 organisms per oil immersion field. All were tested intracutaneously with O.T. (10 mg in controls and 100 mg in O.T. treated), and with heat-killed bacilli, at varying intervals, while 2 from each group received live bacilli intracutaneously 56 days after infection. The O.T.-treated animals gave consistently negative reactions both to tuberculin and to bacilli while the 2 control groups reacted strongly to tuberculin and gave necrotic Koch phenomena with the bacilli.

Microscopically there was a distinct tendency for lesions in the O.T.-treated animals to be less caseous than in control groups. However, in both the O.T. and glycerin treated groups, the extent of the lesions in lungs, livers, and spleens was definitely greater than in the saline controls. Areas of pneumonia containing great numbers of acid-fast bacilli, such as were seen in Series I, were observed in 7 of 18 O.T.-treated animals (living 37, 37, 43, 49, 55, 67, and 68 days). However, 6 of 22 glycerin (living 37, 49, 54, 60, 65, and 73 days) and 3 of 21 saline controls (living 39, 52, and 62 days) also showed the same change. Willis and Woodruff[2] found this lesion in only tuberculin treated animals but their controls received nothing, in contrast to the daily injections of glycerin and saline in our animals. Since in the present experiment hypersensitive controls showed the lesion with marked proliferation of bacilli, and since many of the non-hypersensitive O.T.-treated animals failed to show this lesion, it seems that the lack of hypersensitivity does not play a rôle in producing it.

It seems that some change occurs in certain of the animals receiving daily injections which either inhibits the development of their ability to prevent the multiplication and to destroy the bacilli (resistance, immunity) or else interferes with this mechanism when it has developed. We have considered that the administration of glycerin might be a factor, for Long and Vorwald[3] found that tubercle bacilli grew more profusely in rats receiving glycerin, than in untreated ones. Since 3 of the saline treated pigs showed the enormous numbers of bacilli, glycerin cannot be the only factor. The extraordinary proliferation of bacilli in the lungs in contrast to that in the spleen and liver, and also the fact that the bacilli growing in the lung produce much less necrosis than those introduced intracutaneously, are perplexing phenomena. Further studies of these points and of the antibody titer in the various groups will be reported later.

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[3] Long, E. R., and Vorwald, A. J., *Am. Rev. Tuberc.*, 1930, **22**, 636.

Summary. When guinea pigs were infected with virulent human tubercle bacilli and prevented from becoming hypersensitive by daily subcutaneous injections of O.T., some developed pulmonary lesions teeming with bacilli. However, some of the control animals, demonstrated to be hypersensitive, showed identical lesions. It is, therefore, concluded that the development of lesions of this type is dependent on factors other than a lack of hypersensitivity.

10088 P

Utilization of β-hydroxybutyric Acid by Fed and Fasted Rats.*

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Whenever a substance or some experimental procedure produces a decrease in ketosis, it is of some importance to determine whether this effect is due to a decrease of ketone body production by the liver *(antiketogenesis)*, or to an increase in ketone body utilization by the muscles *(ketolysis)*. Particularly is this significant in considering the influence of glucose administration.[1]

In a series of recent studies, we could obtain no evidence that glucose can accelerate the rate of ketone utilization by the muscles of normal rabbits[2] or by the perfused dog heart.[3] However, the in-

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* Aided by the David May Fund.

† Eli Lilly Fellow.

[1] Mirsky, I. A., Heiman, J. D., and Broh-Kahn, R. H., *Am. J. Physiol.*, 1937, **118**, 290.

[2] Mirsky, I. A., and Broh-Kahn, R. H., *Am. J. Physiol.*, 1937, **119**, 734.

[3] Waters, E. T., Fletcher, J. P., and Mirsky, I. A., *Am. J. Physiol.*, 1938, **122**, 542.

vestigations of Butts and Deuel,[4] Butts,[5] Shapiro,[6] and others lead to the conclusion that glucose is ketolytic in action. In view of this discrepancy, it became of importance to reinvestigate the problem by another approach.

The method employed by Butts and Deuel, by Shapiro, and by some other workers studying the influence of various factors on ketosis, consists in measuring the urinary excretion of ketone bodies after the ingestion of a definite amount of either sodium acetoacetate or of sodium β-hydroxybutyrate. The difference between the amount fed and the amount excreted is interpreted to indicate the amount oxidized. Thus, when Butts and Deuel observed that rats maintained on a stock diet excreted minimal amounts of the fed ketone bodies as compared with fasted rats, or when Shapiro found that the administration of glucose or other glycogenic metabolites resulted in a decrease in the ketone excretion of rats fed diacetic acid, they concluded that glucose produces an increase in the oxidation of the ketone bodies.

In order to evaluate the significance of the above method and the findings obtained with it, we studied the actual disappearance of administered ketone bodies from the entire body of nephrectomized rats. Five sets of female rats were employed (Table I). Two sets (B and E) consisted of animals on a stock diet until a few minutes before the experiment was started. The other 3 sets consisted of animals previously fasted for 24 hours. A bilateral nephrectomy was performed on all the animals under ether anesthesia. Immediately after the completion of the operation, the fed animals (B and E) received an intraperitoneal injection of 100 mg glucose per 100 g of body weight, while one set of fasted animals (C) received a similar injection of glucose, and the other 2 (A and D) an injection of saline. After allowing the animals to recover from the anesthesia, a definite amount of racemic sodium β-hydroxybutyrate per unit of body weight was injected intravenously into one set of fed rats receiving glucose intraperitoneally (E), and one set of fasted rats receiving saline (D), the remaining groups being used for control purposes. A 40-minute period was permitted to elapse before the animals were sacrificed and reweighed. They were then ground up repeatedly in an ordinary meat grinder, and mixed thoroughly to ensure a good mixture. The β-hydroxybutyric acid

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[4] Butts, J. S., and Deuel, H. J., *J. Biol. Chem.*, 1933, **100**, 415.

[5] Butts, J. S., *J. Biol. Chem.*, 1934, **105**, 87.

[6] Shapiro, I., *J. Biol. Chem.*, 1935, **108**, 373.

TABLE I.

Utilization of Intravenously Injected β-hydroxybutyric Acid by Nephrectomized Fed and Fasted Rats in a 40-Minute Period.

Experimental Procedure	No. of Rats	Body wt, g	Surface area, cm²	β hydroxybutyrate injected, m M/K	β-hydroxybutyric acid recovered, m M/K	β-hydroxybutyric acid utilization		
						Actual data, m M/K	Corrected for initial content, m M/K	Corrected for initial content, m M/sq M/hr
A. Fasted-Saline Treated Control	20	143.5 ± 4.7	246.5 ± 4.5	0	0.58 ± 0.065	—	—	—
B. Fed-Glucose Treated Control	10	157.0 ± 4.7	260.0 ± 4.6	0	0.21 ± 0.015	—	—	—
C. Fasted-Glucose Treated Control	10	138.5 ± 3.0	241.5 ± 3.1	0	0.20 ± 0.014	—	—	—
D. Fasted-Saline Treated	20	146.0 ± 3.9	249.0 ± 3.9	8.20 ± 0.030	3.99 ± 0.105	4.21 ± 0.105	4.79 ± 0.124	28.1
E. Fed-Glucose Treated	20	148.0 ± 2.9	251.0 ± 2.9	8.40 ± 0.044	3.49 ± 0.116	4.91 ± 0.116	5.12 ± 0.116	30.1

Deviation from mean = ± standard error of mean.

$$\frac{\text{Difference of mean E — D}}{\text{Standard error of means}} = \begin{array}{l} \text{4.5 (uncorrected)} \\ \text{1.9 (corrected)} \end{array}$$

content of 3 aliquots of each ground-up rat was then determined by a modification of Barnes' method.[7]

Recovery experiments were performed on fed rats sacrificed immediately after the injection of β-hydroxybutyric acid, to determine the efficacy of all the various steps involved. The data thus obtained reveal that the procedure is very reliable since an average recovery of 99.6% was obtained in 5 rats. The details of the experimental procedures will be reported in a subsequent paper.

The data summarized in Table I reveal that if no correction is made for the initial β-hydroxybutyric acid content, a small, though perhaps statistically reliable difference exists between the utilization of β-hydroxybutyric acid by fed rats receiving glucose as compared with fasting rats receiving saline. However, when the spontaneous ketogenesis of fed and fasted rats is taken into account by adding the initial ketone content of the animals to the amount administered, the difference between the fed and fasted rats loses its statistical reliability. Therefore, it is obvious that glucose (or feeding) does not influence the actual utilization of β-hydroxybutyric acid. It is probable from this and other studies still in progress, that the results of Butts and Deuel and of Shapiro must be due to some factor other than ketolysis. The fact that the spontaneous ketogenesis of fasted rats can be decreased by glucose administration suggests the probability that the apparent ketolytic activity reported by the above investigators is due to a cessation of this spontaneous or induced ketogenesis *(antiketogenesis)*.

Summary. There is no significant difference in the rate of utilization of administered β-hydroxybutyric acid by fed or fasted rats receiving glucose. The spontaneous ketogenesis of fasted rats is inhibited by glucose administration and may account for the apparent ketolytic activity reported by others.

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[7] Barnes, R. H., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 352.

10089 P

Blood Iodine in Leukemia.

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Early in the course of a study of the blood iodine levels in various disorders, it became apparent that patients with myeloid leukemia differed from those with leukemia of the lymphatic type in respect to their blood iodine.

An elevation of the blood iodine in a case of lymphatic leukemia was reported by Veil and Sturm.[1] Curtis and his associates have stated[2, 3] that the blood iodine is increased in both lymphatic and myeloid leukemia, but in one case of chronic myeloid leukemia in whom the urinary loss of iodine was studied the blood iodine was approximately normal.[4] Dassen and del Castillo[5] found a high blood iodine in 2 cases of myeloid leukemia. Stevens,[6] using a method similar to the one employed by us found a normal value for the blood iodine in one case of lymphatic leukemia. Summarizing, it is fair to say that the prevailing impression, based on some half dozen cases in the literature, seems to be that the blood iodine is elevated in leukemia regardless of type.

We have made blood iodine determinations according to the method of Trevorrow and Fashena[7] on 17 patients with leukemia. Twelve of these were of the lymphatic type. The results are shown in Table I where the cases are arranged in descending scale according to the blood iodine level. Normal values with this method in our hands range from 3.5 to 6.0γ.

On reference to Table I, it will be noted that in the myeloid cases the blood iodine tends to be normal or below, while in the lymphatic type the blood iodine is normal or high. With one exception (F. de G.) there is no overlapping of the two groups. The average blood iodine for the lymphatic group is 10.2γ as compared with 3.3γ for the myeloid.

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[1] Veil, W. H., and Sturm, A., *Deutsches Arch. f. klin. Med.*, 1925, **147**, 166.

[2] Curtis, G. M., Davis, C. B., and Phillips, F. J., *J. A. M. A.*, 1933, **101**, 901.

[3] Curtis, G. M., Cole, V. V., and Phillips, F. J., *West. J. Surg.*, 1934, **42**, 435.

[4] Curtis, G. M., and Barron, L. E., *Surgery*, 1937, **1**, 92.

[5] Dassen, R., and del Castillo, E. B., *Semana Med.*, 1934, **41**, 1854.

[6] Stevens, C. D., *J. Lab. and Clin. Med.*, 1937, **22**, 1074.

[7] Trevorrow, V., and Fashena, G. J., *J. Biol. Chem.*, 1935, **110**, 29.

TABLE I.
Blood Iodine Values in Leukemia.

Patient	Sex	Age	Type of leukemia	Blood Iodine γ/100 cc
W.B.	M	56	lymphatic	17.9
M.S.	M	69	,,	17.8
A.J.	M	64	,,	14.1
A.D.	F	46	,,	13.2
E.C.	M	56	,,	11.7
R.T.	M	73	,,	10.5
J.H.	M	61	,,	9.3
C.B.	M	56	,,	8.6
F.deG.	M	27	myeloid	7.4
S.T.	F	63	lymphatic	5.2
A.A.	M	75	,,	4.7
I.L.	M	55	,,	4.6
J.L.	M	54	,,	4.3
J.P.	M	31	myeloid	3.8
N.M.	F	56	,,	2.5
A.D.	F	52	,,	1.7
M.P.	M	42	,,	1.3

It seemed possible that the variation noted might be due to a difference in the iodine content of the white blood cells. However, in a few instances, when we made a crude attempt to separate the red cells from the white cells and plasma by means of a separating funnel, no difference could be demonstrated between the iodine content of whole blood and of the white cell-plasma fraction.

In 2 cases of lymphosarcoma the blood iodine was found to be high and it seems possible that this may be of some diagnostic value. The blood iodine was slightly elevated in 2 cases of Hodgkin's disease and normal in 2 others.

10090 P

Effect of Histamine Pretreatment upon Adrenalectomized Rats.

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There are many features of histamine "shock" and the "intoxication" of adrenal insufficiency which are similar. Karady has found[1] that the incidence and severity of surgical shock or histamine shock in the rat may be reduced by previous treatment with histamine. We have found that pretreatment with increasing doses of histamine will increase the resistance of adrenalectomized rats to certain procedures to which they are ordinarily very susceptible.

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[1] Karady, St., *Am. J. Physiol.*, 1938, **121**, 194.

Bilateral nephrectomy causes a marked decrease in the survival time of rats from which both adrenals have been removed.[2, 3] Sixteen male albino rats weighing from 144-193 g were divided into 2 groups with an average weight of 170 g. The histamine-treated group was given a 10% suspension of finely ground crystals of histamine dihydrochloride in olive oil so that its action might be more protracted.[4] Ten mg were administered subcutaneously daily for 6 days, followed by 20 mg per day for 8 days more, a total of 220 mg per rat. At this time the 2 groups still weighed about the same but the histamine-treated rats appeared to be in the poorer condition. Sixty hours after discontinuing the histamine treatment the adrenals and the kidneys were removed from all of the rats. In the histamine-treated group the cortex of the adrenal gland had become hypertrophied so that the adrenals of this group weighed 31% more than those of the controls. The survival time of the 2 groups of rats was quite at variance with their appearance. The untreated controls survived for from 28 to 41 hours with an average of 34.6 hours. The histamine-treated group, on the other hand, lived from 43 to 73.5 hours with an average of 56.8 hours, an increase in the average survival time of 64%.

Adrenalectomized animals are usually very sensitive to the effect of ingested potassium salts.[5] Pretreatment with histamine before the removal of the adrenal glands raises the resistance of adrenalectomized rats to potassium salts. Twelve adult male rats were divided into 2 groups, 6 controls (266 g) and histamine-treated (262 g), of approximately equal weight. The rats of the histamine-treated group were given 360 mg histamine dihydrochloride per rat over a period of 17 days, starting with 10 mg daily for 5 days, then 20 mg for 8 days, 40 mg for 1 day and 100 mg for 1 day, all in aqueous solution. Twenty-four hours after the last injection both groups were adrenalectomized. As in the first experiment there was marked hypertrophy of the adrenals in the histamine-treated group, apparently limited to the cortex. Nine, 19, 22, 24, and 26.5 hours after adrenalectomy both groups received 2% KCl by stomach tube in a dose of 1 cc per dcm^2 of body surface, averaging 94 mg of KCl per

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[2] Ingle, D. J., and Kendall, E. C., *Am. J. Physiol.*, 1936, **117**, 200.

[3] MacKay, E. M., Bergman, H. C., and MacKay, L. L., *Am. J. Physiol.*, 1937, **120**, 83.

[4] Keeney, E. L., *Bull. Johns Hopkins Hosp.*, 1938, **62**, 227.

[5] Allers, W. D., Nilson, H. W., and Kendall, E. C., *Proc. Staff Meet. Mayo Clinic*, 1936, **11**, 283.

dose for the control group and 92 mg per dose for the histamine-treated group. The rats in the control group survived for 24.7-32 hours with an average of 27.5 hours after the operation while the rats pretreated with histamine survived 32-56 hours with an average of 41.0 hours after the operation.

The bearing of these results on the interpretation of adrenal function proposed by Selye[6] is of interest. He concluded that one of the most important functions of the adrenals is to increase the resistance to "alarming stimuli" such as variable temperature, excessive muscular exertion, toxic drugs, etc. The adrenals are conceived to play an important rôle in the first stages of such adaptations, while later resistance is acquired by the peripheral tissues; the stimulus ceases to be alarming and the adrenal hormones are no longer required for adaptation. He further assumes that the symptoms of the alarm reaction are due to liberation of a toxic metabolite such as histamine and that the adrenal detoxifies this substance. Our data is the first experimental evidence directly supporting Selye's theory. The activity of the histamine destruction mechanism of the organism is greatly reduced after adrenalectomy[7] and there is every reason to believe that this mechanism is much more active following pretreatment with increasing doses of histamine. It is of importance that adrenalectomized dogs can acquire a tolerance toward potassium salts[8] and Selye has found[9] that histamine adaptation is not lost after adrenalectomy.

The decrease in survival time of adrenalectomized rats produced by nephrectomy[2, 3] may well be due to an important decrease in the histamine-destroying activity of the organism, for the renal tissue has by far the highest histaminase content of any tissue[10, 11] and this organ destroys the most histamine *in vivo*.[7]

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[6] Selye, H., *Science,* 1937, **85**, 247.

[7] Rose, B., and Browne, J. S. L., *Am. J. Physiol.,* 1938, **121**, 175.

[8] Kendall, E. C., and Ingle, D. J., *Science,* 1937, **86**, 18.

[9] Selye, H., *Arch. internat. Pharmacodynamie.,* 1937, **55**, 431.

[10] McHenry, E. W., and Gavin, G., *Biochem. J.,* 1932, **26**, 1365.

[11] Edlbacher, S., and Zeller, A., *Helv. Chim. Acta.,* 1937, **20**, 717.

10091

Adrenal Cortical Hormone and Ant. Pituitary Extract on Carbohydrate Levels in Fasted Hypophysectomized Rats.*

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Hypophysectomized rats are unable to maintain their carbohydrate stores during fasting as do normal animals.[1] Certain anterior pituitary extracts are capable of preventing completely the changes found in short fasts, such as 8 hours. In longer fasts, however, while muscle glycogen stores are held at normal or supernormal levels, the liver glycogen and blood glucose are not maintained by this treatment.[2] This difference in the response of liver and muscle has led to the suggestion that more than one factor may be concerned in the maintenance of carbohydrate levels in the fasted hypophysectomized rat.[3]

Because of the possibility that the adrenal cortex may mediate some of the effects of the anterior pituitary on carbohydrate metabolism, the relationship of the adrenocorticotropic substance of the A.P. and of the adrenal cortical hormones to the factor or factors mentioned above has recently been investigated. That the maintenance of muscle glycogen stores is possible in the absence of the adrenal cortex was early found;[2] and Bennett, working in this laboratory, has recently presented much additional evidence that the factor maintaining muscle glycogen (the "glycostatic" factor) is not the adrenocorticotropic factor.[4] Bennett also showed that adrenocorticotropic preparations on chronic administration (that is, with maintenance or repair of the adrenal cortex) could effect consider-

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The authors wish to express their gratitude to Dr. Herbert M. Evans for his advice and assistance in carrying on this investigation.

Acknowledgment is herewith made of services rendered by the Works Progress Administration, Project No. 8877A.

† Porter Fellow of the American Physiological Society, 1937-38.

[1] Russell, J. A., Proc. Soc. Exp. Biol. and Med., 1936, **34**, 279; Russell, J. A., and Bennett, L. L., *Am. J. Physiol.*, 1937, **118**, 196.

[2] Russell, J. A., and Bennett, L. L., Proc. Soc. Exp. Biol. and Med., 1936, **34**, 406.

[3] Russell, J. A., *Endocrinol.*, 1938, **22**, 80.

[4] Bennett, L. L., *Endocrinol.*, 1938, **22**, 193.

able increases in the liver glycogen and blood sugar levels of fasted hypophysectomized rats.[5] From a consideration of these facts, it seemed possible that 2 pituitary factors affecting the carbohydrate stores in these animals were (1) a "glycostatic" substance acting directly on the muscle glycogen, and (2) the adrenocorticotropic hormone affecting also the liver glycogen and thence blood sugar levels. Evidence supporting this suggestion is here presented.

In the experiments reported here (Table I), hypophysectomized rats 2-4 weeks post-operative were fasted 24 hours and given during this time either Kendall's crystalline Compound B from the adrenal cortex‡ or a standard alkaline extract of beef anterior pituitary[2] or a combination of the 2 substances. When these experiments were partly completed, the report of Long and Katzin appeared with data on the effects of cortical extract on the carbohydrate levels of fasted hypophysectomized rats.[6] The figures are entirely confirmatory of those we obtained using Kendall's crystalline Compound B alone. Marked increases in liver glycogen content were produced; and blood sugar levels were normal or a little above. However, in neither the series of Long and Katzin nor in our several groups were the muscle glycogen values maintained at fully normal levels. In spite of the use of high doses of the hormone and the production of extremely high liver glycogen levels, normal muscle glycogens were not obtained except in isolated cases. These results are entirely parallel to those which Bennett found after treatment of the rats for some days with adrenocorticotropic extracts.

On the other hand, the effects of acute treatment with anterior pituitary extracts, of which an example is given here, are very different from those of either the adrenocorticotropic hormone or of the adrenal cortex substance. Although approximately 6 times the dosage required to produce a maximal effect was used, the action of the anterior pituitary extract was evident solely on the muscle glycogen stores; in this case the muscle glycogen levels were maintained completely. With the combination of the 2 types of treatment—the crystalline Compound B with injection of the anterior pituitary extract—the stores of muscle glycogen and of liver glycogen were both much higher than with either type of treatment alone (compare Groups 3 and 5, Table I). While some synergism may have occurred in this case, it seems more probable that the effect was additive, the maintenance of glycogen in the muscles by the anterior

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[5] Bennett, L. L., PROC. SOC. EXP. BIOL. AND MED., 1937, **37**, 50.

‡ We wish to thank Dr. E. C. Kendall for this substance.

[6] Long, C. N. H., and Katzin, B., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 566.

TABLE I.
Carbohydrate Levels of Hypophysectomized Rats Fasted 24 Hours with and without Adrenal Cortical Hormone* and Anterior Pituitary Extract.

	No. of rats	Blood Glucose mg %	Liver Glycogen mg %	Muscle Glycogen mg %
1. Untreated Rats	10	42 (30-54)	13 (8-20)	350 (250-400)
2. A.C.H., 1 mg total dose; injection every hr during last 8 hr of fast	5	76 (53-106)	137 (46-236)	382 (290-437)
3. A.C.H., 3 mg total dose; fed during 24 hr fasting period	5	72 (57-94)	97 (41-156)	453 (417-475)
4. A.C.H., 3 mg total dose; injected every hr during 24 hr or fed during first 16 hr, injected every hr during last 8 hr	5	90 (73-106)	917 (37-1950)	480 (448-512)
5. A.C.H., 3 mg total dose, fed or injected during 24 hr fasting period, + 1.5 cc A.P.E.† during fast	7	78 (64-103)	440 (179-1370)	629 (530-722)
6. A.P.E., alone (1.5 cc during fast)	6	46 (36-64)	11 (7-13)	565 (550-612)
7. Normal Rats, untreated	15	72 (65-85)	25 (15-60)	525 (490-550)

* Compound B, crystalline, from Kendall.
† Standard alkaline extract of anterior lobes of beef pituitary.

pituitary extract allowing greater accumulation of glycogen in the liver under the influence of the cortical hormone.

The cortical hormone seems able to cause an increase in the glycogen content of fasted animals in which the carbohydrate levels are low before the injections are started, as found by Long and Katzin in a small series and in our experiments (Group 2). It is noticeable in the latter series that whereas the liver glycogen was supernormal and the blood sugar level also up, the muscle glycogen content was scarcely affected. Apparently, then, this substance acts directly on the liver, affecting the muscle glycogen level only more slowly and probably indirectly.

From the results presented here it appears that at least 2 factors with different functions act together to prevent the fall in carbohydrate levels which ordinarily takes place in fasted hypophysectomized rats. The factor affecting the muscle glycogen content directly, the "glycostatic" factor, appears to act independently of the adrenal cortex and to be associated with none of the other known anterior pituitary hormones. It does not appear to affect liver glycogen or blood sugar values under the usual conditions, and probably acts by suppressing carbohydrate oxidation.[7] Kendall's Compound

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[7] Fisher, R. E., Russell, J. A., and Cori, C. F., *J. Biol. Chem.*, 1936, **115**, 627.

B, or a physiologically similar substance produced when the adrenal cortex is stimulated by adrenocorticotropic extracts of the anterior lobe, causes increases in liver glycogen and blood sugar levels in these animals, only more slowly and incompletely restoring the muscle glycogen levels. When the cortical hormone and a glycostatic extract of the anterior lobe are administered simultaneously, the separate effects of each on liver and muscle are apparent.

10092

Sensitized Pupillary Dilator and Facial Muscles as Indicators of Sympathetic and Parasympathetic Substances in Blood.*

MORRIS B. BENDER. (Introduced by G. Shwartzman.)

From the Laboratories of the Mount Sinai Hospital, New York City.

It is well known that section of the cervical sympathetic renders the pupillary dilator fibers sensitive to circulating adrenaline. Therefore, any discharge of epinephrine into the blood stream would be indicated by greater dilatation in the denervated than in the normal pupil. The degree of dilatation varies with the concentration of adrenaline or sympathin in the blood and with the sensitivity of the radial fibers of the iris, which in turn depends on the completeness of denervation. This pupillary reaction may be used as an indicator for sympathetic hormonal activity.

An indicator for circulating parasympathetic substance (acetylcholine) may be prepared by sectioning the facial nerve (Bender[1]). This renders the denervated facial muscles sensitive to acetylcholine. In the presence of small amounts of acetylcholine, slow contraction in the paralyzed muscles may be seen. By repeated denervations the sensitivity of the reaction may be maintained for long periods.

On sectioning the superior cervical ganglion on one side and the facial nerve on the opposite, indicators for both sympathetic and parasympathetic substances were obtained. Monkeys, cats, dogs and rabbits were used. The operative procedures were simple, and the two sections were carried out in one period. Usually the left facial nerve and the right cervical sympathetic were cut.

The sensitization phenomenon appeared on the sympathectomized

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* This investigation has been aided by a grant from the Josiah Macy, Jr., Foundation.

[1] Bender, M. B., *Am. J. Physiol.*, 1938, **121**, 609.

side 1 to 8 days after operation, and on the parasympathectomized side 4 to 14 days following the facial denervation. In the cat, dog, and rabbit, spontaneous tremors in the whiskers on the side of facial denervation appeared at the time the denervated facial muscles first became responsive to acetylcholine. Thus, by noting whether there were tremors in the whiskers, it was possible to predict when the denervated muscles were sensitive to acetylcholine.

Following this, the sensitization properties of these structures (pupil, nictitating membrane and face) continued to increase. The sensitivity was determined by repeated intravenous injections of adrenaline, acetylcholine with or without eserine, and combinations of these drugs. At the maximum point of sensitization it was found that (a) the minimal amount of adrenaline necessary to produce minimal dilatation of the denervated pupil was 0.25 gammas injected into the saphenous vein of a cat weighing 3.2 kg; (b) the minimal amount of acetylcholine necessary to produce a minimal contraction in the denervated facial muscles was 2.0 gammas injected into the saphenous vein of a monkey weighing 2.4 kg. In the eserinized animal, much lower concentrations (*e. g.*, 0.2 gammas) of acetylcholine were effective in producing facial contractions. The minimal dose in each species varied with the body weight. The determinations were made with the animals under nembutal or ether anesthesia. Cats were most sensitive indicators for sympathetic substances and monkeys acted best as indicators for circulating parasympathetic substances.

The minimal contractions were seen best in the ears and whiskers of the cat, dog and rabbit and in the eyelid and lips of the monkey. Any movement in these structures may be recorded photographically on a moving film.

The preparation was used for the following studies: (1) Under strong emotion, not only did the denervated pupil dilate, but the denervated facial muscles on the opposite side contracted, indicating that sympathetic and parasympathetic substances were discharged. In the cat, the pupillary dilatation was observed to be more conspicuous of the two, while in the monkey, the contraction in the face was the more obvious.

(2) Convulsions induced with metrazol produced dilatation of the denervated pupil which lasted for 5 and 20 minutes in the monkey and cat respectively. The effect, however, was more prominent in the cat. There was no manifest change in the denervated facial muscles.

(3) Injections of large amounts of insulin U 50-200 caused, in some monkeys, a slow and prolonged though not marked contraction

in the denervated face. This was not easily observed in other species.

(4) Injection of 4.0 gammas of acetylcholine into the general circulation of the cat resulted in a definite contraction in the denervated face within 7 seconds and dilatation of the denervated pupil in 17 seconds. The dilatation of the pupil must have been due to circulating adrenaline or sympathin. The latter supposition is explained by the fact that acetylcholine stimulated the adrenergic system (after 7 seconds—circulation time) liberating adrenaline into the blood stream which reached the denervated pupil 10 seconds later. The phenomenon of acetylcholine stimulating the formation of adrenaline was well illustrated in the cat.

TABLE I.

Cat weighing 2800 g. Left facial nerve and right superior cervical ganglion with sympathetic trunk were resected 10 days previously. Nembutal 1.4 cc was given intraperitoneally. Eserine 0.5 mg was given intramuscularly in order to inhibit cholinesterase activity in the cat used as the indicator. Atropine 1.0 mg was injected intramuscularly to counteract the muscarine effects of the injected acetylcholine. Two cc volumes of mixed acetylcholine and human blood serum were used. Several such mixtures were used in the determinations.

Serum concentration 25%.

Acetylcholine concentration 0.01% (1 cc equals 100.0 gammas).

10:45 A.M.—Threshold dose for minimal contraction—3.0 gammas into saphenous vein.

Aliquots injected into saphenous vein		Duration of serum mixed with Ach. in seconds	Results: Minimal contraction in ear	% hydrolyzed of 200 gammas
Vol/cc	Amt/gamma			
0.1	10	30	+	
0.1	10	40	±	
0.1	10	60	0	60%
0.2	20	60	++	
0.2	20	80	±	
0.2	20	110	0	
0.3	30	110	±	
0.3	30	180	0	92.5%
0.4	40	90	+	
0.4	40	120	+	
0.4	40	160	±	
0.5	50	160	+	
0.8	80	200	0	
1.0	100	220	±	96%
1.4	140	240	0	
1.6	160	240	0	
2.0	200	180	+	
2.0	200	255	±	98%

11:11 A.M.—Threshold dose: 4.0 gammas ± into saphenous vein.

Cerebrospinal fluid showed similar cholinesterase activity, but at a much slower rate.

The reverse of this, *i. e.,* adrenaline stimulating the secretion of acetylcholine was also found, but thus far only in 2 monkeys. Injections of large amounts of adrenaline, 400 to 1000 gammas, caused a pronounced dilatation of the denervated pupil followed in 2 to 3 minutes by a slow contracture in the denervated face.

(5) The denervated face was also used as an end point in the determinations of rates of hydrolysis of acetylcholine by blood, and other body tissues and fluids. The great advantage of this preparation is that small (physiologic) amounts of acetylcholine could be used in these determinations and the indicator reaction is almost specific for acetylcholine.

Summary. (1) A preparation is described in which sympathomimetic and parasympathomimetic substances could be detected in the blood. (2) Any discharge of either or both of these substances under various physiologic states was indicated by dilatation of the denervated pupil or by contraction in the facial muscles respectively. (3) Under emotional stress, the cat exhibited predominantly sympathetic activity while the monkey showed parasympathetic activity in their denervated structures. (4) The denervated facial muscles were used as indicators of end points in the determinations of rates of hydrolysis by sera and cerebrospinal fluids.

10093 P

A Bacterial Enzyme Which Converts Creatine into its Anhydride Creatinine.*

René J. Dubos and Benjamin F. Miller.

From the Hospital of the Rockefeller Institute for Medical Research, N. Y., and the Department of Medicine, University of Chicago.

We have previously described the preparation from two bacterial species (NC and HR) of enzymic systems that are highly specific for the decomposition of creatine and creatinine.[1, 2] These enzymic systems function only under aërobic conditions and convert creatine and creatinine into urea, ammonia, and other compounds. We have now observed that the washed cells of the NC bacterial species are capable of converting creatine into creatinine under anaërobic conditions; the creatinine so produced can then be decomposed by the same bacterial suspension when the conditions are rendered aërobic.

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* This investigation was aided by a grant to one of the authors, B. F. Miller, from the John and Mary R. Markle Foundation, New York City.

[1] Miller, B. F., and Dubos, R. J., Proc. Soc. Exp. Biol. and Med., 1936, **35**, 335.

[2] Dubos, R. J., and Miller, B. F., *J. Biol. Chem.*, 1937, **121**, 429.

The new enzyme has been obtained in aqueous solution, free of the cellular structure, by killing the cells with cold acetone, or with toluol and chloroform.

This enzyme appears to exhibit a high degree of specificity for creatine, since it does not convert the closely related compound glycocyamine into glycocyamidine, nor does it change hydantoic acid into its anhydride, hydantoin. The enzyme does not produce creatine or creatinine from arginine, a possible precursor of creatine in the animal body. Methyl guanidine and acetic acid, component parts of creatine, are not synthesized into the latter compound by the enzyme.

The enzyme is not produced when the bacterial cells are grown in a medium which does not contain creatine; it seems, therefore, to belong to the group of "adaptive enzymes" which are produced only in the presence of their specific substrate.

The conversion of creatine into creatinine offers an opportunity for the study *in vitro* of (a) the enzymatic production of a biologically important cyclic compound from an aliphatic one, and (b) the enzymatic combination of an amino and a carboxyl group to form the important CO-NH linkage.

10094

Changes in the Ant. Pituitary Gland of Rats with Experimental Goiter.

ROSE COHEN NELSON AND JOSEF WARKANY. (Introduced by Glenn E. Cullen.)

From the Children's Hospital Research Foundation and the Department of Pediatrics, University of Cincinnati.

The relationship of the thyroid to the pituitary gland has been explored by various methods. Earliest mention of this relationship is found in the description of the hypophysis[1] in pathologic conditions of the thyroid gland. Large, pale cells with nuclei poor in chromatin were noted in the anterior hypophyses in cases of thyroid deficiency.[2] Similar observations were made in dogs with spontaneous goiter.[3] Following thyroidectomy, the disappearance of acidophils and appearance of large chromophobes or basophils—frequently designated as thyroidectomy cells—have been described.[4] Marine, Rosen and Spark[5] recently found an increase in the size of all the glandular cells and a decrease of the acidophilic granules in the anterior pituitary of rabbits maintained on a goitrogenic diet. Addition of iodine or desiccated thyroid to the diet of these animals prevented such changes or restored the appearance of the anterior pituitary gland to normal.

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[1] Niepce, B., *Traite due goitre et du cretinisme*, Paris, J. B. Bailliere, 1851.

[2] (a) MacCallum, W. G., and Fabyan, M., *Bull. Johns Hopkins Hosp.*, 1907, **18**, 341; (b) Schilder, P., *Virchows Arch. f. path. Anat.*, 1911, **203**, 246; for review see (c) Brauchli, H., *Frankfurt Ztschr. f. Path.*, 1925, **31**, 450.

[3] Romeis, B., *Virchows Arch. f. path. Anat.*, 1924, **251**, 237.

In rats reared on a goitrogenic diet we have found changes in the anterior pituitary gland which appear similar to those described in pathological human glands and following thyroidectomy. The Steenbock and Black[6] rachitogenic diet No. 2965 was found to be goitrogenic by Krause and Monroe.[7] In order to protect the rats against rickets, irradiated ergosterol in olive oil was added to their diet. Every tenth day an amount equivalent to 60 International Units was fed by pipette. The addition of vitamin D did not interfere with the regular production of hypertrophy and hyperplasia of the thyroid gland[8] and made it possible to keep the animals on the diet for a long time. Twenty-one males and 7 females were put on the diet after weaning; they were sacrificed after 2, 3, 4, 5, or 12 months. The growth of the rats was considerably retarded and they did not reproduce. Control animals were kept on Bill's modification of the Steenbock stock diet.[9]

In every experimental animal the thyroid showed hypertrophy and hyperplasia. Fig. 1, A and B, shows the obvious difference between the thyroid of an experimental animal and a control. The pituitary changes were not marked in animals which had been on the diet for 2 months or less. After this period changes were observed which were much more definite in the males than in the females. In sections stained with hematoxylin and eosin, the presence of groups of large and pale cells gives the anterior lobe a spongy appearance visible under low power magnification (Fig. 2). These groups are usually seen in the center of the gland, rarely in the lateral

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[4] (a) Rogowitsch, N., *Beitr. z. path. Anat. u. z. allg. Path.*, 1888-89, **4**, 453; for review see (b) Zeckwer, I. T., Davison, L. W., Keller, T. B., and Livingood, C. S., Jr., *Am. J. M. Sc.*, 1935, **190**, 145.

[5] Marine, D., Rosen, S. H., and Spark, C., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 803.

[6] Steenbock, H., and Black, A., *J. Biol. Chem.*, 1925, **64**, 263.

[7] Krauss, W. E., and Monroe, C. F., *J. Biol. Chem.*, 1930, **89**, 581.

[8] (a) Thompson, J., *J. Nutrition*, 1932, **5**, 359; (b) Levine, H., Remington, R. E., and von Kolnitz, H., *J. Nutrition*, 1933, **6**, 325.

[9] Bills, C. E., Honeywell, E. M., and MacNair, W. A., *J. Biol. Chem.*, 1928, **76**, 251.

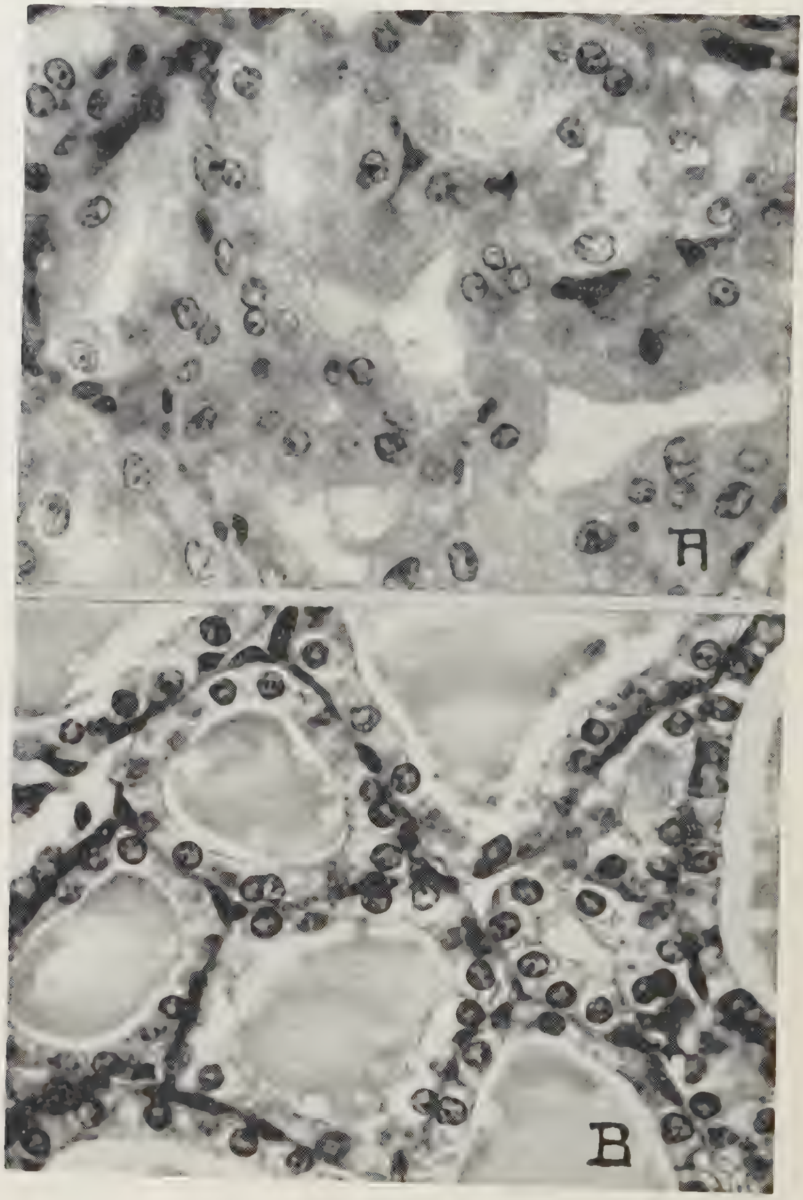

FIG. 1.
A. Hyperplastic thyroid gland of male rat 6Y after 4 months on goitrogenic diet. (800×)
B. Normal thyroid gland of control male rat 8YC after 4 months on standard diet. (800×)

parts of the anterior lobe. The cells appear in nests of 5 to 20 and show well-defined cell boundaries in many cases. Under higher magnification (Fig. 3 A), it becomes obvious that the abnormal size of the cell is due to an increase of the amount of cytoplasm, which appears finely granular about the nucleus and vacuolated about the periphery. The nucleus is not markedly changed in size but to some extent is lacking in chromatin. This description tallies closely with that of thyroidectomy cells or goiter cells. The relationship of these

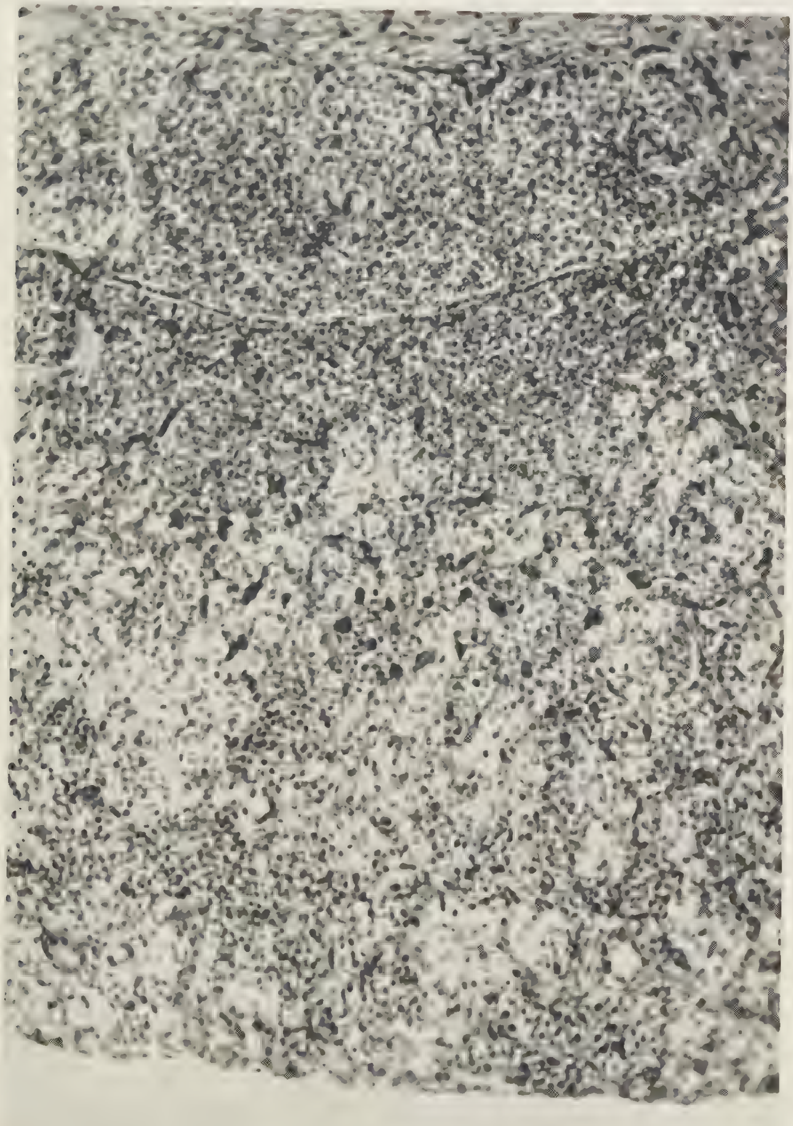

FIG. 2.
Anterior and median lobe of the pituitary gland of rat 6Y showing nests of large cells. (200×)

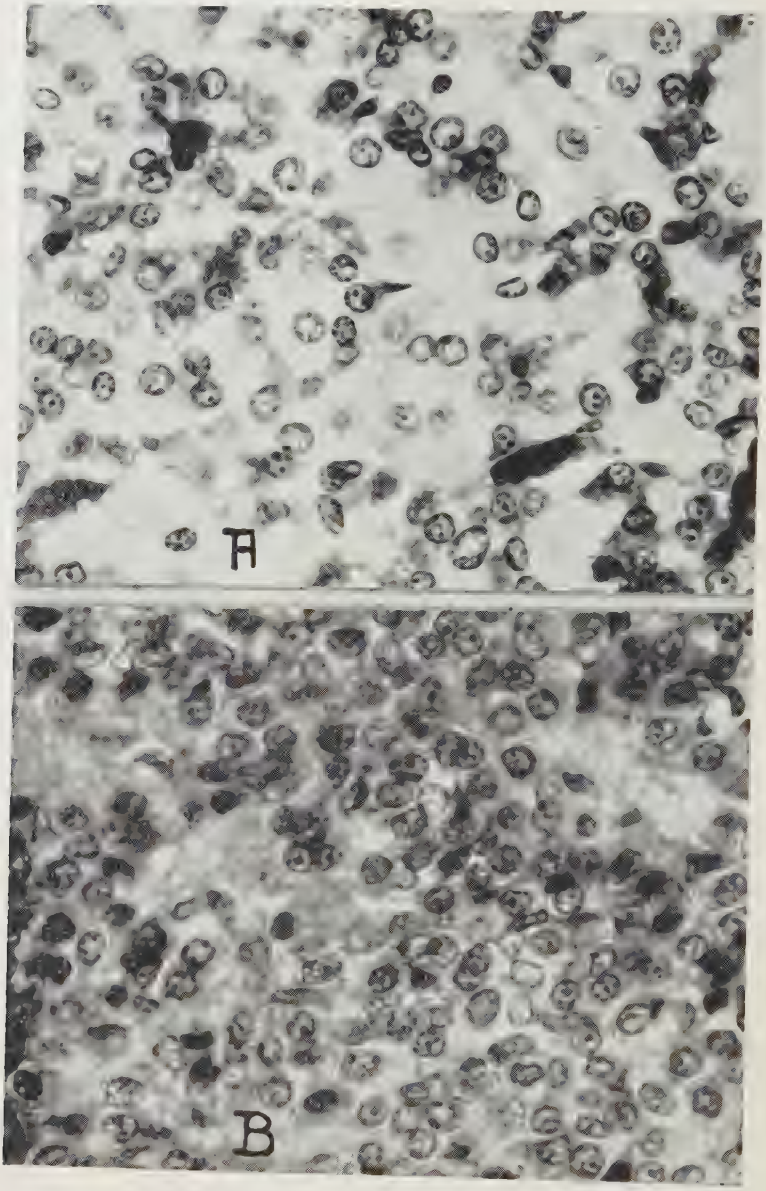

FIG. 3.
A. Section of anterior pituitary shown in Fig. 2. (800×)
B. Section of anterior pituitary of control male rat 8YC. (800×)

cells to castration or pregnancy cells is still under discussion and it would appear premature to make any statement as to their functional significance. Among 12 males which were on the diet longer than 2 months, 11 showed definite changes while only 2 of the 7 females presented similar findings under the same condition. In an additional group of 9 females put on the diet after maturation, only 2 showed the large cells described above. The reproductive system was examined in all animals. The rats which were 2½ to 3 months of age and had been on the diet 2 months or less showed a retardation of the reproductive organs. Among the males who remained on the diet for a longer period, 11 showed only slight retardation on histologic examination; one appeared definitely retarded. The females reared on the diet showed delayed maturity at the age of 5 or 6 months, while those put on the diet after maturation presented normal reproductive organs. In order to rule out the absence or insufficiency of vitamins E and A as the cause for the changes found, wheat germ oil and carotene oil were added to the diet of some of the experimental animals. These additions had no effect upon the results.

10095

The Standardization of Diluted Tuberculin.

W. E. Bunney and R. Y. Gottschall. (Introduced by C. C. Young.)

From the Biologic Products Laboratory, Michigan Department of Health, Lansing.

In developing the method for standardization of tuberculin described in this paper, we were seeking one superior to any hitherto reported, in two respects: (1) a method that would be accurate within at least 20% instead of the 40 and 60% accuracy hitherto available; (2) an accurate method so sensitive that it could be used to standardize tuberculin ready diluted 1-10,000. We required such a method so that tuberculin ready diluted in the diluent previously described[1] could be tested with precision after dilution, and just before distribution, so that the material used by the physician in the field could be of known potency.

We had been comparing tuberculin of our manufacture with the International Standard tuberculin of the League of Nations for a number of years by injecting a 1-10,000 dilution of the standard and the unknown tuberculin in each arm of 100 individuals. Variations in the results indicated that this was far from an accurate method of comparison. Also carefully run experiments in which a 1-10,000 dilution, and 70, 80, or 90% of this concentration of a tuberculin equivalent to the International Standard, were injected into the 2 arms of sensitive individuals, showed that a 20% difference in 2 dilutions could not be determined reliably by this method. These injections were made at the Ingham Sanatorium, Lansing, by Dr. G. C. Stucky, resident physician, to whom we are indebted for this part of the work. The injections were made as unknowns and read independently by 2 workers as unknowns. As much difference was found between the 90 and 100% reactions as between the 70 and 100%, and the 80% reactions averaged larger than the 100%.

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[1] Gottschall, R., and Bunney, W. E., *J. Immunol.*, 1938, **34**, 103.

The use of guinea pigs as test animals has advantages. They are more readily available and multiple injections can be used, so that a series of dilutions of 2 tuberculins can be compared on the same animal . The method of testing diluted old tuberculin used by Parish and Okell,[2] and by Douglass and Hartley,[3] in their studies on the properties and stability of "synthetic" old tuberculin was found to be inadequate for testing first test-dose material (1-10,000 dilution), since too few of their animals were sensitive enough to react satisfactorily to a 1-10,000 dilution of tuberculin. With their method we found but 2 of 15 animals to be suitable for titrating 1-10,000 tuberculin. The method eventually found most satisfactory is a modification of that devised for the standardization of old tuberculin on tuberculous guinea pigs as described by Lewis and Aronson[4] and Eagleton and Baxter.[5] This method was suggested by Calmette and de Potter[6] as the most valuable for standardization of tuberculin, and as modified appears to fulfill the requirements we had adopted as our goal.

Sensitization of Animals. White 700 to 1400 g guinea pigs, from 6 to 24 months old, are sensitized by injecting in the groin, 0.5 mg (wet weight) of a 4-week culture of Saranac Lake strain of *Myco. tuberculosis,* H37, grown on Dorset's synthetic agar medium, or Petragnani's medium. Old healthy discarded breeders or animals which have been released from other biological tests are particularly well suited for this work. The suspension of organisms is prepared by thoroughly triturating the weighed bacilli in a tube by means of a glass rod, adding a drop of warm liquefied gelatin and emulsifying until an homogeneous mixture is obtained. Sufficient sterile Clark and Lubs phosphate buffer, pH 7.2 to 7.4 is slowly added with constant stirring to make a final concentration of 1.0 mg per milliliter.

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[2] Parish, H. J., and Okell, C. C., *J. Path. and Bact.*, 1929, **32**, 51.

[3] Douglass, S. R., and Hartley, P., *Tubercle,* 1934, **16**, 105.

[4] Lewis, P. A., and Aronson, J. H., *Am. Rev. Tuberc.*, 1923, **7**, 404.

[5] Eagleton, A. J., and Baxter, E. M., *Brit. J. Exp. Path.*, 1923, **4**, 289.

[6] Calmette, A., and de Potter, *Ann. de l'inst. Pasteur,* 1926, **40**, 353.

The animals are sufficiently sensitive at the end of 4 weeks and are then tested by injecting intracutaneously 0.1 ml of a 1-10,000 dilution of a standard tuberculin. Those showing a 2+ reaction (10 to 20 mm induration) 48 hours later are used for testing old tuberculin at the 1-10,000 level. We have found that at least 90% of the animals sensitized in this manner can be used for titrating 1-10,000 tuberculin and some of our animals have reacted to a 1-320,000 dilution of a tuberculin equivalent to the International Standard. Parish and O'Brien[7] found the "sensitivity limit" of their animals to be 1-8000 but several of their animals reacted to a 1-25,000 dilution of tuberculin equivalent to the International Standard.

Description of the Test. 1-20,000, 1-40,000, and 1-80,000 dilutions of tuberculin are prepared in saline from the first strength or 1-10,000 dilution of the unknown to be tested for potency. A similar series of freshly prepared dilutions from 1-10,000 to 1-80,000 and another series at 80% of these values or 1-12,500, 1-25,000, 1-50,000, and 1-100,000 are made. One-tenth ml of these 12 dilutions are injected across the back[5] of 3 closely clipped animals, each series being placed at a different position on each animal to avoid as far as possible variation in size and intensity of reactions due to location alone. The highest dilution of each series is always placed on the left side farthest from the backbone and the other dilutions in order across the back. The injection-scheme is as follows:

Guinea pig 1	Guinea pig 2	Guinea pig 3
100% series	Unknown	80% series
80% "	100% series	Unknown
Unknown	80% "	100% series

The skin is first marked off with a wax pencil, the first series being placed well behind the nape of the neck and the last series in the lumbar region. Reactions on the nape of the neck or too far toward the rump of the animal are frequently unreliable. When the second test dose or 1-100 dilution is titrated this tuberculin and the standard are also diluted to the 1-10,000 level and injected as in titrating the 1-10,000 dilution.

After 24 and 48 hours the reactions are read and an attempt is made to differentiate the 100% from both the 80% and the unknown series before locating their positions from the records. If the 100% series has been correctly differentiated from the unknown or the 80% series the assumption is made that a difference of 20% is readable on this animal and the unknown is compared with the 80 and 100% series and read as being 100%, 80 to 100%, or less than 80%

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[7] Parish, H. J., and O'Brien, R. A., *Brit. Med. J.*, 1935, **1**, 1018.

of the standard. In case the 80% and 100% reactions are similar but the unknown reactions are weaker the value of the unknown must be less than 80%. Those animals in which all 3 series are similar are discarded as unreadable since a variation in strength of 20% is not distinguishable. Readings are made 24 and 48 hours after injection.

One-ml tuberculin syringes and 28-gauge needles are used for making injections. Because of the "tenacity" of tuberculin[7] the syringes are treated with strong chromic-acid cleaning solution for 2 to 4 days to remove traces of tuberculin not destroyed by ordinary cleaning as are also the Bureau of Standards pipettes used for making the dilutions.

Occasionally animals on preliminary test with 1-10,000 tuberculin show an area of induration of 20 to 30 mm diameter, with a central necrotic area. It is then necessary to use dilutions either from 1-20,000 to 1-160,000, or 1-40,000 to 1-320,000 (depending on the sensitivity) instead of 1-10,000 to 1-80,000. Large reactions do not appear to give as fine a differentiation as smaller reactions. The highest dilution should give a diameter of redness of from 4 to 8 mm if the test is to be accurate.

This method has been found valuable for routine testing of ready-diluted tuberculin before distribution, and for following the stability of diluted and undiluted tuberculin. It seems to be more accurate than the method in common use in which Okell and Parish[8] found a difference of 40% between 2 tuberculins to be just barely differentiable.

After the completion of this work it was thought desirable to determine the accuracy of the tests by titrating a number of unknown percentages of 1-10,000 dilutions of tuberculin. Ten out of 50 percentages of 1-10,000 dilutions ranging from 60 to 100% of the standard 100% or 1-10,000 dilutions were therefore selected by a disinterested worker and tested by one of us who was ignorant of the values of the tuberculin. The tests were made in the manner previously described, 3 animals being used for each unknown. Results are shown in Table I. While the number of unknown titrations is small it is apparent from these figures that the assigned values were all within 20% of the actual values.

We feel safe in assuming that a 1-10,000 dilution of tuberculin which has lost potency can be detected if the loss is greater than 10%, and that the actual loss can be estimated correctly within 20%. The use of a larger number of animals for each titration would prob-

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[8] Okell, C. C., and Parish, J. H., *Brit. J. Exp. Path.*, 1927, **8**, 170.

TABLE I.

Actual and Assigned Values of Unknown Dilutions of Tuberculin. Diluted 1-10,000

Unknown No.	Actual value of unknown	Assigned value
1	70%	70-80%
2	70	60-70
3	80	90
4	100	90
5	60	70
6	80	90
7	90	90
8	90	80-90
9	80	70-80
10	70	70-80

ably still further increase the sensitivity of the test.

In the development of the test it was found essential to compare a series of dilutions of the 2 tuberculins as described so that the series of dilutions could be evaluated as a whole. This because in our hands it was impossible to differentiate with assurance between 2 dilutions differing from each other by 30 or even 40%, if single injections of each dilution were made in the same guinea pig. As a corollary of this fact it was found that multiple injections of a single dilution into some guinea pigs could give reactions varying as much as 40% in size. Not until we used a series of dilutions causing a 2+ reaction in the lowest dilution, and a diameter of redness of 4 to 8 mm in the highest dilution, was an accurate comparison of 2 tuberculins possible, and then only if the 2 series of reactions were compared as a whole.

The minimal number of 3 guinea pigs was adopted for the test because it was found in a series of comparisons on 54 guinea pigs, that two-thirds of them gave readily differentiated reactions to 1-10,000 dilution and 80% of this value. In most of the remaining third the reactions to the 2 dilutions were identical. If a minimum of 3 pigs previously proved sensitive to the 1-10,000 dilution is used it practically never happens that all 3 animals are unsuitable for differentiating a 20% difference in potency.

Summary. A skin-test for determining the potency of diluted tuberculin is described. It can be used to accurately standardize tuberculin ready diluted to the 1-10,000 level. With this method the results are accurate within 20%, consequently a diluted tuberculin which is but 80% of the original strength can be detected with little difficulty. The method is inexpensive since discarded animals can be used without altering the sensitivity of the test. The lower limit of accuracy seems to be well within the limit of error of routine human group-testing.

10096

Colorimetric Assay of Urinary Androgens.

Ralph B. Oesting.* (Introduced by Bruce Webster.)

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In earlier communications by the author[1, 2] on the colorimetric assay of urinary androgens no data were given on crystalline preparations of the pure hormones normally found in the urine. These data are of value in interpreting colorimetric work and are given here.

Crystalline androsterone (M.P. 180°u.c.) dehydroisoandrosterone (M.P. 135°u.c.) and testosterone (M.P. 152°u.c.) were obtained and weighed quantities made up into 60% alcohol. Color unit and capon assays were carried out on these preparations with the results recorded in Table I.

TABLE I.
Color Unit and Capon Comb Growth Equivalents of Crystalline Androgens.

Substance	Gamma equivalent to 1 color unit	Range	Gamma equivalent to 1 capon unit	No. of assays
Androsterone	90	78-100	8-12	10
Dehydroisoandrosterone	90	80-105	90-130	8
Testosterone	75	70-84	8-15	10

(One capon unit = the total dose distributed into 5 daily applications of equal size to the combs of at least 2 capons which will cause an average increase in comb area of 15%.)

The biological activity of a color unit from urine extracts was found to be approximately equal to 10 gamma of pure androsterone. This value varies as is evident from the data of Table I of our earlier report.[2] The range in comb growth for a color unit is of necessity wide. This is to be expected since:

1. The chemical reaction used is non-specific.
2. The various androgens found in urine are not always excreted in the same proportions.
3. The variations in the capon assay contribute to this factor and the number of capons used in this work was of necessity small.
4. There are probably other non-active androgen-like color-producing substances (such as undoubtedly result from the hydro-

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* General Education Board Fellow.

[1] Oesting, R. B., Proc. Soc. Exp. Biol. and Med., 1937, **36**, 524.

[2] Oesting, R. B., and Webster, B., *Endocrinology*, 1938, **22**, 307.

lysis treatments) which have evaded attention simply because of their lack of activity.

From the above data we see that one color unit of pure androsterone is represented by 90 gamma of the crystalline material, while one color unit of urine extract is equivalent in biological activity to only 10 gamma of androsterone. This is an expected finding, but one that must be remembered when using the colorimetric method as a guide to quantitative studies.

10097

Effect of Estrogen Injections on Lactogen Content of Female Rat Pituitary.

R. P. REECE.* (Introduced by W. C. Russell.)

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Earlier work[1] has demonstrated that pituitary glands from sexually mature ovariectomized rats injected with estrogen contained more lactogen than glands from sexually mature ovariectomized non-treated rats. Ovariectomy itself, however, decreased the lactogen content of the rat pituitary gland.[1] We were interested, therefore, in ascertaining the influence of estrogen injections on the lactogen content of pituitary glands from animals with their ovaries intact.

Three series of rats, with 20 rats in each series, were employed in this study. The animals designated as experimental animals in the third series, however, are the same animals as those designated experimental animals in the second series. Rats were paired on the basis of body weight and they were all fed the same stock diet. Daily vaginal smears were made from injected animals, and from the control animals during the last 4 days of the experimental period. All animals were sacrificed the day following the last day of injection, their pituitaries and other endocrine organs removed and weighed. The lactogen content of the pituitary glands was determined by the assay method employed by Reece and Turner.[2]

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* Journal Series Paper of the New Jersey Agricultural Experiment Station, Department of Dairy Husbandry.

[1] Reece, R. P., and Turner, C. W., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 283.

[2] Reece, R. P., and Turner, C. W., *Mo. Agr. Exp. Sta. Res. Bul.*, 1937, 266.

Series I. Ten rats that had been running normal estrous cycles were injected subcutaneously daily for 15 days with 200 i.u. of estrogen (Progynon-B†). The control animals were normally cyclic rats. Vaginal smears of the injected rats revealed a complete cornification of the vaginæ on the 2nd day of injection. During the remainder of the injection period the vaginal smears consisted mainly of cornified cells with leucocytes appearing at irregular intervals. The estrogen injections elicited an increase in the weight of the pituitary gland, a decrease in ovarian weight, and no significant change in thyroid or adrenal weight. The pituitary glands from the injected rats contained 40% more lactogen than did glands from non-injected animals. This increase in lactogen content was due entirely to increased pituitary size because the lactogen concentration was decreased.

Series II. Ten normally cyclic rats were injected subcutaneously daily for 15 days with 1000 i.u. of Progynon-B. The control rats were normally cyclic animals. The vaginæ of the injected rats were completely cornified on the 2nd or 3rd day of injection and cornified cells were present in the vaginal smears throughout the injection period. Beginning on the 6th day and continuing throughout the injection period nucleated epithelial cells and/or leucocytes were present in the majority of the vaginal smears. In this connection it should be mentioned that Wolfe[3] has reported that the vaginal smears of rats injected with 1000 i.u. of Progynon-B daily for 8 to 15 days revealed a complete cornification of the vaginæ for the first 3 to 5 days of the experiment. This condition did not persist throughout the experiment. On autopsy, as has been reported by other investigators,[3, 4] the ovaries of the injected rats contained corpora lutea which were similar in size and appearance to the corpora lutea of rats sacrificed in late pregnancy.

Estrogen injections induced an increase in pituitary size but not as great as that observed in Series I. The ovaries of 7 out of 10 experimental animals were heavier than those of the control animals. There was no significant difference in the weights of the thyroid and adrenal glands of the control and experimental animals. The pituitary glands from the injected animals contained 28% more lactogen than did those from non-injected animals, and, as was observed in Series I, this increased hormone content was due entirely to increased pituitary size, for there was a slight decrease in hormone concentration.

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† The Progynon-B used in this study was kindly supplied by Dr. E. Schwenk of the Schering Corporation.

[3] Wolfe, J. M., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 757.

[4] Hohlweg, W., *Klin. Wchnschr.*, 1934, **13**, 92.

TABLE I.
Effect of Estrogen Injections on Lactogen Content of Female Rat Pituitary.

No. of Animals	Physiological condition	Avg body wt beginning of exp. g	Avg body wt when sacrificed g	Avg pituitary wt, mg	Avg thyroid wt, mg	Avg adrenal wt, mg	Avg ovarian wt, mg	Bird units per pituitary gl	Bird units per mg pituitary tissue	Bird units per 100 g body wt
10*	Normally cyclic rat	149	160	8.9	15.2	46.7	59.1	7.93	0.89	4.96
10†	200 i.u. Progynon-B/day for 15 days	152	161	15.7	17.4	45.7	47.5	11.10	0.71	6.89
10*	Normally cyclic rat	160	167	7.9	16.3	42.1	58.9	12.6	1.59	7.54
10†	1000 i.u. Progynon-B/day for 15 days	161	159	13.3	19.3	41.5	64.4	16.1	1.21	10.13
10*	Ovariectomized + 1000 i.u. Progynon-B/day for 15 days	162	160	21.4	14.1	42.9		14.3	0.67	8.94
10†	1000 i.u. Progynon-B/day for 15 days	161	159	13.3	19.3	41.5	64.4	14.1	1.06	8.87

* Control Animals.
† Experimental Animals.

Series III. For control animals 10 normal cyclic rats were ovariectomized and injected subcutaneously daily for 15 days with 1000 i.u. of Progynon-B. The experimental animals in this series were the same as those in Series II.

The vaginal smears of the control animals indicated cornification of the vaginæ on the 2nd day of the injection period. The smears, during the remainder of the injection period, consisted largely of cornified cells. An occasional smear, however, contained large nucleated epithelial cells and/or leucocytes.

Of most interest in this series is the observation that each one of the pituitary glands from the control animals was heavier than the gland from its experimental mate. On the average, the pituitaries from the control animals weighed 21.4 mg and those from the experimental animals weighed 13.3 mg. This observation is somewhat surprising inasmuch as Brooksby[5] reported that 500 gamma of progesterone daily enhanced the action of estrogen in causing macroscopic enlargement of the pituitary.

Each one of the thyroid glands from the experimental animals was heavier than the thyroid gland from its control mate. There was no difference in adrenal weight between the 2 groups of animals.

The lactogen content of the pituitary glands from the control and experimental animals was nearly identical when average figures are considered. When the pairs are considered separately, 6 of the glands from the control animals contained more lactogen than the glands from the experimental animals, one was equal, and 3 contained less lactogen.

The results are summarized in Table I.

Conclusions. The injection of Progynon-B into rats with their ovaries intact augmented the lactogen content of their pituitary glands. The pituitary glands from ovariectomized rats injected with 1000 i.u. of Progynon-B daily for 15 days were significantly larger than those from rats with their ovaries intact and similarly treated, but the lactogen content per pituitary was similar.

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[5] Brooksby, J. B., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 832.

10098 P

A Flocculation Phenomenon with Human Sera and Suspensions of the Virus of Epidemic Influenza.

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Neutralization and complement-fixation tests with the virus of epidemic influenza have been described and studied rather extensively but no agglutination, precipitation, or flocculation reaction has as yet been reported. Theoretically such reactions should be demonstrable, provided a sufficient quantity of virus is contained in the antigen.[1] Attempts to purify and concentrate the virus by ultracentrifugation in this laboratory have not in general yielded suitably reactive antigens. During the course of investigation it was observed, however, that a flocculative phenomenon could be obtained consistently with certain human sera and crude suspensions of lungs of mice infected with influenza virus, the reaction being especially marked with sera of patients convalescent from epidemic influenza. It is the purpose of this report to describe the conditions under which the reaction occurs and to discuss briefly some of the results.

Methods. Mice of the Swiss strain are inoculated intranasally with 1 to 2% suspensions of lungs of mice infected with PR8 strain of influenza virus. At the time of appearance of visible areas of pulmonary consolidation—approximately 40 to 50 hours after inoculation—the mice are sacrificed and the lungs ground with alundum in meat-infusion broth (pH 7.8) to form a 10% suspension by weight. After light centrifugation equal volumes of supernatant fluid and serum are mixed in small tubes and incubated at 37°C. After 1 hour's incubation the tubes are shaken lightly and the degree of flocculation is recorded in the usual manner. Frequently flocculation does not occur until the tubes are shaken but once the reaction takes place a precipitate settles to the bottom of the tubes in large coarse floccules. Meat-infusion broth is not essential in the preparation of the antigen and can be replaced by M/150 phosphate buffer pH 7.2 or 0.4% NaCl solutions. However, antigens prepared with broth are more stable than those prepared with either buffer or 0.4% saline.

Results. The majority of sera from patients convalescent from epidemic influenza produced strong flocculative reactions with infected-mouse-lung antigen while sera from the same individuals during the acute stage of the disease, on the whole, failed to react. On the other hand, convalescent sera from an epidemic of non-influenzal upper respiratory infection which occurred in the western United States during 1936[2] failed to produce the reaction as did all but one of a number of sera from convalescent cases of pneumococcal pneumonia. Furthermore, antigens prepared in a similar manner from normal mouse-lung failed to react with any of the sera.

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[1] Merrill, M. H., *J. Immunol.*, 1936, **30**, 169.

Strong sera induce good flocculation in dilution of 1:8 or 1:16, but with undiluted serum the antigen is effective in dilution not greater than 1:2 or 1:4.

Heating of serum or antigen at 56°C for 1 hour destroys the ability of either to induce the reaction.

Table I summarizes some of the results obtained with sera from several respiratory infections. It is to be noted that the convalescent serum from one case of streptococcal infection of the throat showed no increased neutralizing or complement-fixing antibodies for influenza virus, yet evoked a fairly good flocculative reaction, whereas, none of the 3 reactions was positive in the presence of "acute" serum from the same individual. The results of the neutralization-tests with sera from the California epidemic of 1936 are recorded as "no change," indicating no increase in antibody-titer in convalescent as compared with acute serum. The technic of the neutralization test employed with these sera differed somewhat from that usually employed and so the serum-titers are not strictly comparable with the other tests.

Specificity of the reaction. There are several indications that the reaction is a specific one between the influenza virus and its antiserum. These are (1) the occurrence of the reaction in the presence of serum from convalescent influenzal patients and not in the presence of serum from the same individuals during acute stages of the disease; (2) the failure of convalescent sera from cases of pneumococcal pneumonia and other respiratory infections to induce the reaction; (3) the occurrence of the reaction with the serum of convalescent influenzal patients in the presence of antigen prepared from lungs of mice infected with influenza virus and lack of the reaction in the presence of antigens prepared from normal mouse-lung. There is, however, some reason to doubt the specificity of the reaction. First, it occurs occasionally in the presence of convalescent serum from individuals who were considered not to have had influenza, as in the one case of hemolytic-streptococcal infection of the throat. Second, the intensity of the reaction obtained with

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[2] Francis, T., Jr., *Am. J. Pub. Health,* 1937, **27**, 211.

TABLE I.

Flocculative Reaction with Suspensions of Influenza Virus and Different Sera.

			Highest dilution of serum giving positive reaction			
			Flocculation with suspensions of			
Serum		Disease	Infected lung	Normal lung	Neutralization	Complement-fixation
RM	Acute	Influenza	0	0	1-30	0
	Conv.		1-2	0	1-240	1-128
EW	A	”	±	0	1-6	1-16
	C		1-8	0	1-50	1-64
MW	A	”	0	0	1-10	1-8
	C		1-8	0	1-60	1-512
VH	A	Strept. throat	0	0	1-30	1-8
	C		1-4	0	1-30	1-8
LH	A	” ”	1-4	0	1-100	1-32
	C		1-8	0	1-100	1-32
EB	A	Sinusitis	0	0	0	0
	C		0	0	0	0
RA	A	Pneumococcal pneumonia	0	0		
	C		0	0		
LR	A	Pneumococcal pneumonia	0	0		
	C		0	0		
JS	A	Pneumococcal pneumonia	0	0		
	C		0	0		
AM	A	Pneumococcal pneumonia	0	0		
	C		1-2	0		
WJ	A	Non-influenzal upper respiratory infection California 1936	0	0	No change*	
	C		0	0		
LR	A		0	0	” ”	
	C		0	0		
RE	A		0	0	” ”	
	C		0	0		
RG	A		0	0	” ”	
	C		0	0		
HF		No recent infection	1-2	0	1-10	1-16
TM			1-8	±	1-10	1-8
RD			1-8	±	1-2	1-8
AM			0	0	1-10	1-32
LV89			1-8	0	1-2	0
LV132			1-2	0	1-2	1-8

* Indicates no increase in neutralizing antibodies of convalescent serum as compared with acute serum.

various antigens does not parallel the viral content of the antigen as determined by infectiousness for mice. The intensity of the reaction does parallel, however, within limits, the increased weight of the lung which takes place during infection with influenza virus. Third, in certain fractionation-experiments reactions of a somewhat different character have been observed in the absence of virus.

It may be that the antigen is a combination of active and altered virus, and that infectivity for mice is not an accurate measurement of the amount of virus that can react with the serum. On the other hand, it may be that the flocculating antigen is some substance other than the virus which appears in the mouse-lung during infection, similar perhaps to the nonspecific precipitinogen in yellow fever described by Hughes.[3] The problem is being investigated further.

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[3] Hughes, T. P., *J. Immunol.*, 1933, **25**, 275.

10099

Effect of Sulfanilamide upon the Viability of Meningococci in Spinal Fluid.

ERWIN NETER.

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The investigations of Branham and Rosenthal,[1] Buttle, Gray, and Stephenson,[2] McPherson Brown,[3] Proom,[4] Rosenthal, Bauer, and Branham,[5] have established the curative value of sulfanilamide and related compounds in experimental meningococcal infections of animals. In man, favorable results in the treatment of meningococcal meningitis by means of sulfanilamide (either alone or in conjunction with antimeningococcal serum) were reported by numerous observers.[6-17] In a recent communication,[18] it was reported that sulfanilamide may exert a bacteriostatic action upon meningococci present in spinal fluid of patients with meningococcal meningitis. When such spinal fluids were incubated with sulfanilamide, growth of meningococci failed to take place, while the number of microörganisms in a specimen without sulfanilamide showed a marked increase. In the following, experiments are being reported dealing with the action of sulfanilamide upon the viability of meningococci.

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[1] Branham, S. E., and Rosenthal, S. M., *Publ. Health Rep.*, 1937, **52**, 685.

[2] Buttle, G. A., Gray, W. R., and Stephenson, D., *Lancet*, 1936, 1286.

[3] McPherson Brown, Th., *Bull. Johns Hopkins Hosp.*, 1937, **61**, 272.

[4] Proom, H., *Lancet*, 1937, 16.

[5] Rosenthal, S. M., Bauer, H., and Branham, S. E., *Publ. Health Rep.*, 1937, **52**, 662.

[6] Schmidt, W., *Deutsche med. Wchnschr.*, 1936, **62**, 881.

[7] Schwentker, F. F., Gelman, S., and Long, P. H., *J. Am. Med. Assn.*, 1937, **108**, 1407.

[8] McIntosh, R., Wilcox, D. A., and Wright, F. H., *J. Pediat.*, 1937, **11**, 167.

[9] Mitchell, A. G., and Trachsler, W. H., *J. Pediat.*, 1937, **11**, 183.

[10] Brennemann, J., *J. Pediat.*, 1937, **11**, 238.

[11] Bernstein, S. S., *J. Pediat.*, 1937, **11**, 198.

The experiments were carried out in the following manner: Spinal fluid was obtained from patients with meningococcal meningitis prior to treatment. It was taken and kept under sterile precautions. Ten drops of spinal fluid were mixed with equal amounts of sulfanilamide in various concentrations, ranging from 0.8% to 0.0064%. The preparation used was Prontylin, Winthrop, repurified for injection, and was dissolved in physiological saline solution. The spinal fluid-sulfanilamide mixtures were then incubated at 37°C. After various periods of incubation, subcultures were made on 30% ascitic-chocolate-agar plates. The plates were incubated at 37°C in a jar containing about 10% CO_2. The resulting growth of meningococci was noted. Table I gives the results of such an experiment.

TABLE I.
Action of Sulfanilamide upon Viability of Meningococci in Spinal Fluid.

	Concentration of sulfanilamide %	Growth of meningococci on ascitic-chocolate-agar at 37°C after treatment with sulfanilamide for—							
		A 5 min		B 45 min		C 3 hr		D 24 hr	
		a	b	a	b	a	b	a	b
1.	0.4	++	++	+	++	0	+	0	0
2.	0.08	++	++	++	++	0	++	0	0
3.	0.016	++	++	++	++	++	++	0	0
4.	0.0032	++	++	++	++	++	++	+	++
5.	0	++	++	++	++	++	++	++	++

a	After 18 hr at 37°C.
b	After 48 hr at 37°C.
++	Marked growth
+	Slight growth.
0	No growth.

It may be seen from this table, that treatment for 5 minutes of meningococci in spinal fluid, with various concentrations of sulfanila-

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[12] Basman, J., and Perley, A. M., *J. Pediat.*, 1937, **11**, 212.

[13] Carey, B. W., *J. Pediat.*, 1937, **11**, 202.

[14] Zendel, J. F., and Greenberg, D., *New York State J. Med.*, 1937, **37**, 1744.

[15] Willien, L. J., *J. Am. Med. Assn.*, 1938, **110**, 630.

[16] Eldahl, A., *Lancet*, 1938, 712.

[17] Crawford, T., and Fleming, G. B., *Lancet*, 1938, 987.

[18] Neter, E., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 37.

mide did not inhibit the growth of this microörganism. When the treatment with sulfanilamide was carried out for 3 hours, the growth of meningococci on subculture was retarded. While growth of meningococci from spinal fluid containing 0.016% sulfanilamide, or less, occurred after 18 hours' incubation at 37°C, growth from spinal fluid containing 0.08% sulfanilamide, or more, appeared only after 48 hours' incubation On the other hand, when spinal fluid was treated with sulfanilamide in concentrations of 0.016% or more for 24 hours, no growth was obtained on subculture. Substantially the same results were obtained with spinal fluids of 3 other patients with meningococcal meningitis.

It follows from these experiments that the effect of sulfanilamide upon the viability of meningococci in spinal fluid depends upon the concentration of the drug as well as upon the period allowed for its action. The experiments furthermore show that the small quantities of sulfanilamide, present in the spinal fluid-sulfanilamide mixtures used for the inoculation of the medium cannot be responsible for the delay and inhibition of growth, since meningococci grew profusely within 18 hours from spinal fluid-sulfanilamide mixtures being kept for short periods only.

In order to elucidate whether the above described effects of sulfanilamide upon meningococci present in spinal fluids of patients with meningococcal meningitis depends upon the presence of leukocytes, parallel experiments were carried out with meningococci suspended in infusion-broth. It was found that treatment with sulfanilamide may result in loss of viability of meningococci in the absence of leukocytes.

In summary, sulfanilamide may have the following action upon meningococci: Firstly, in the presence of sulfanilamide, the growth of menigococci may be inhibited. Secondly, previous treatment of meningococci with sulfanilamide may result in the retardation of their growth on subculture or in the loss of their viability, depending upon concentration of the drug as well as upon the period allowed for its action.

10100

Preparation of Stomach Pouch Without Interruption of Vagal Supply.*

FRANKLIN HOLLANDER AND EDWARD E. JEMERIN.
From the Laboratories of the Mount Sinai Hospital, New York City.

As demonstrated previously,[1] the ventral and dorsal gastric vagus trunks in the dog run symmetrically with each other along the lesser curvature of the stomach. Their secondary branches pass across the stomach, transversely to its long axis (Fig. 1). The Pavlov pouch technic was based upon the erroneous anatomical concept that only the ventral trunk runs along the lesser curvature, whereas the dorsal trunk courses along the greater curvature. In consequence, the bulk of the vagal supply to the pouch was unwittingly sacrificed by the operative procedure. To determine whether there exists a physiological counterpart to this anatomical deficiency, it is necessary to compare the Pavlov type of pouch with one in which the vagi are anatomically undisturbed. Such a pouch has been successfully made in 6 animals by the following technic:

An upper abdominal incision is made slightly to the left of the midline, to avoid the falciform ligament. The stomach is delivered through the wound, milked out, and a single gastric clamp is applied parallel to its long axis and across both fundus and pylorus, as high up as the exposure will permit (Fig. 1). The abdomen is then packed off in the usual manner. The anterior vagal trunk may be seen accompanying the right and left gastric arteries along the lesser curvature of the stomach; its secondary branches accompany the secondary vascular branches (not shown) as they run transversely to the long axis of the stomach. The initial incision in the stomach starts at A on the greater curvature, about 1 inch proximal to the junction of the pyloric antrum with the body of the stomach. From this point it runs about half way across both the anterior and posterior stomach walls in a direction parallel to the secondary vagal branches. The incision is made through the entire thickness of both gastric walls, terminating at B on the anterior wall and at C on the posterior. Preliminary ligation of the gastro-epiploic vessels or their branches is not necessary since these can usually be separated bluntly from the greater curvature for a sufficient distance

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* Supported in part by a grant from the Friedsam Foundation.

[1] Jemerin, E. E., and Hollander, F., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 139.

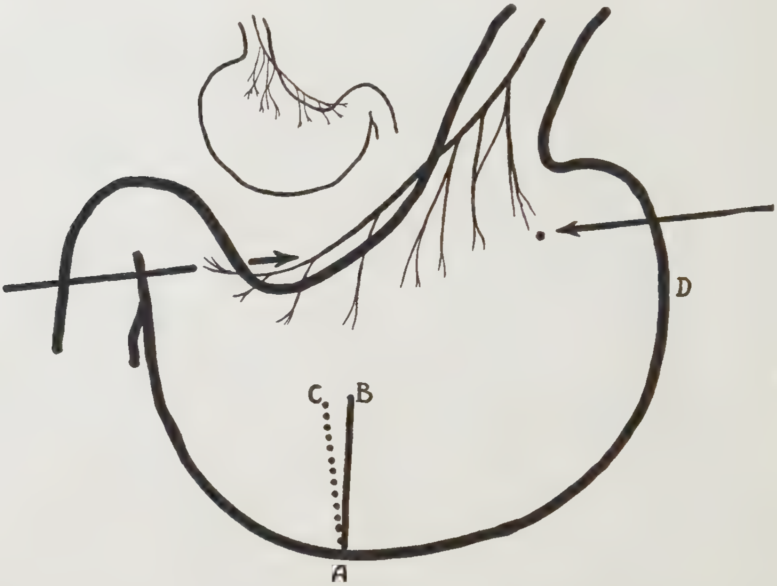

FIG. 1.

Diagram of anterior surface of dog's stomach showing direction of initial incisions in relation to course of anterior vagus trunk and branches. Insert shows symmetrical position of posterior vagus.

A-B: Initial anterior gastric wall incision.
A-C: Corresponding posterior gastric wall incision.
D: Proximal, fundal limit of pouch.
Arrows: Approximate position of gastric clamp.

to permit the incision to be effected. To demarcate the fundal, proximal limit of the pouch, point D on the greater curvature is now selected by projecting a line from B (or C) to the greater curvature in the direction of the long axis of the stomach. The point is marked by a double threaded suture, both ends of which are passed from without inward so that traction may later be made by the suture from within the lumen of the stomach. The portion of the stomach to be converted into the pouch, about ⅓ of the whole, is now outlined by the points A, B, C, and D.

In order to complete the separation of pouch and stomach, B and C are next connected to D by incision of the mucosa and part of the thickness of the submucosa. This is effected as follows. The stomach is turned inside out along the line B-D, this being facilitated by traction on the suture at D. The assistant's finger is placed under the serosal aspect of the line B-D, exposing the mucosal side; the mucosal corrugations are eliminated by applying the proper degree of tension. With a very sharp scalpel, the mucosa is incised along the line B-D, and without releasing the position or tension, flaps of mucosa about three-eighths of an inch wide are dissected back on either side of the incision by sharply splitting the submucosa. Care must be taken not to traumatize or shred the mucosa, and not to dissect deeper than the submucosa. The same process is repeated along C-D, thus completing the mucosal incision from B to D to C without affecting seromuscular continuity. Hence, the branches of the vagus nerve pass uninjured from the main stomach into the pouch.

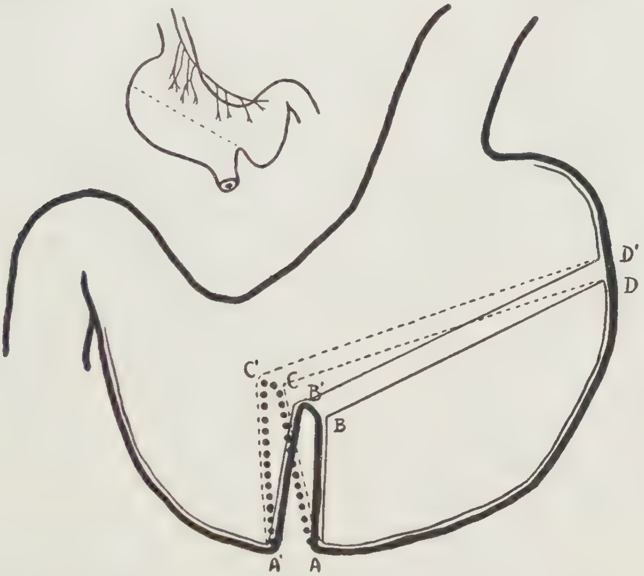

FIG. 2.

Diagram illustrating stomach with incisions completed. Fine lines represent mucosa; heavy lines sero-muscular layers. Insert shows completed pouch (posterior aspect) with uninterrupted vagal supply.

AB: Edge of incision through all gastric layers; anterior surface; pouch side.
AC: Edge of incision through all gastric layers; posterior surface; pouch side.
A′B′ and A′C′: Corresponding incision edges; gastric side.
BD: Edge of incision through mucosa only; anterior surface; pouch side.
CD: Edge of incision through mucosa only; posterior surface; pouch side.
B′C′ and C′D′: Corresponding incision edges; gastric side.

For descriptive purposes, points on the stomach side of the incision will now be called A′, B′, C′, etc., corresponding to A, B, C, etc., on the pouch side (Fig. 2). Beginning at D, flap D-B is now sutured to flap D-C, using a running inverting suture of fine silk or Pagenstecher linen. In order to prevent excessive purse-stringing, the suture is interrupted every few stitches by locking or tying. On the stomach side, D′-B′ is similarly sutured to D′-C′. This results in the separation of the stomach from the pouch by a double-layered septum of mucous membrane. Sutures should be small and closely spaced. They should pass through the submucosa only and must not pierce the flap, as this predisposes to future communications between the pouch and the stomach. Particular care must be taken in laying down this suture line since the extensive length of the septum increases the likelihood of its perforation. Such perforation occurred several times during our early operative efforts, but rarely thereafter—as indicated by the absence of ingested carmine from the pouch contents.

Closure of the stomach is now completed by suturing B′-A′ to C′-A′. Similarly, B-A is sewed to C-A, leaving, however, an opening at A for the pouch mouth. We prefer having this fistula no larger than sufficient to admit a 16 French catheter.

A small counter-incision is made in the abdominal wall, to the left of the main wound, at the site where the pouch can be brought up with the least possible tension. Skin and fascia are incised, while muscle is separated bluntly. After incising the peritoneum, the pouch mouth is brought up through this counter-incision and sutured to peritoneum, fascia and skin. The small size of the pouch stoma, plus the natural tonus of the bluntly separated musculature through which it has been brought out upon the abdominal wall, usually result in sufficient sphincter action so that gastric juice will be retained within the pouch. Thus, retention experiments, unaccompanied by the disadvantages of an indwelling catheter, can be performed as previously described by Hollander and Cowgill.[2]

The main wound is closed in layers. A soft, rubber, 16 French catheter must be inserted into the pouch at the end of the operation and left there for several days. Otherwise, tension due to accumulated blood and gastric secretion may lead to rupture of the septum or to perforation into the free peritoneal cavity.

Fig. 2 insert is a diagram of the completed pouch, illustrating its uninterrupted vagal supply. Experiments are now in progress, comparing the psychic response of such pouches with that of typically constructed Pavlov and Heidenhain pouches.

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[2] Hollander, F., and Cowgill, G. R., *J. Biol. Chem.*, 1931, **91**, 151.

10101

Cytoplasmic Changes Induced in the Walker Rat Carcinoma 256 by X-radiation of Different Intensities.

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A comparison of the cytoplasmic changes induced in the Walker rat carcinoma 256 by different types and dosages of radiation revealed disorganization in the Golgi apparatus and little, if any, change in the mitochondria.[1] In that series of experiments it was noted that the enlargement of the Golgi region in the disorganization process seemed to bear some relationship to the dosage of irradiation, the greater the dosage the more marked the enlargement and density of the blackened mass. A subsequent series of experiments was carried out to test the effect of a variation in the milli-amperage with all other factors unchanged as nearly as possible from the earlier series.

The disorganization of the Golgi apparatus and mitochondrial changes have been reported by several workers based on studies on the effects of radiation.[2-5] Heilbrunn's[6] review of the literature in Duggar's Biological Effects of Radiation shows that radiation has a destructive effect on mitochondria. It is interesting to note that Emmel[7] noted a period of fragmentation of the Golgi apparatus and a darker staining osmic impregnated cytoplasmic area in the cells of the Ringer perfused frog's kidney.

In general the same procedure was followed as in the previous experiments. Rats bearing 10-day-old tumors were used for irradiation. Only the rats bearing uniformly growing, hard, nonnecrotic tumors were used. The tumor growth characteristics are well known and came from the laboratory's continuously inbred strain of Sloniker white rats. No premature death of animals or change in the growth characteristics of the tumor, such as undue necrosis, occurred in this series of experiments.

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[1] Fogg, L. C., and Warren, S., *Am. J. Cancer*, 1937, **31**, 567.

[2] Guyer, M. F., and Claus, P. E., *Anat. Rec.*, 1934, **61**, 57.

[3] Bartelmetz, G. W., and Bensley, C. M., Special Cytology, edited by E. V. Cowdry, Paul B. Hoeber, New York, 1932, **3**, 1525.

[4] Ludford, R. J., Tenth Scientific Report, Imperial Cancer Research Fund, 1932, p. 125.

[5] Nahm, L. J., *J. Morph.*, 1933, **54**, 259.

[6] Heilbrunn, L. V., and Mazia, D., Biological Effects of Radiation, edited by B. M. Duggar, McGraw-Hill, New York, 1936, **1**, 625.

[7] Emmel, V. M., *Anat. Rec.*, 1938, **70**, 371.

The following is an outline of the experiments:

Walker 256—10 day tumor.

No. rats	Dosage	Voltage
6	800 r at 15 MA	200 KV
6	800 r at 20 MA	200 KV
6	800 r at 25 MA	200 KV

For each series irradiated tumor bearing rats from each of the above series were killed at intervals of 2, 4, 6, 9, 12, 18 hours after onset of radiation. At each interval animals were killed by a blow to avoid the possible effect of anesthesia on tissue. As before the tumor was excised and small fragments of non-necrotic (*i. e.,* pearly white, somewhat hard and opaque) tissue were placed in fixative. Modified Mann-Kopsch method was used for revealing the Golgi apparatus and both Schridde and Regaud methods were used for the mitochondria. For this tissue these methods were adequate in that they revealed well stained cells.

A study of the effect of the radiation on the cytology of the cell confirms that of our earlier work.[1, 8] In general, it causes a cessation of mitosis for a limited period, a variability in the ease of demonstration of mitochondria and a specific series of changes brought about in the Golgi apparatus. With a dosage of 800 r mitosis was completely retarded for a period less than 24 hours. The mitochondria underwent very little morphological change. The portion of the cell containing the Golgi net underwent the general sequence of changes, namely, the normal compact net, the spreading, the fragmentation, the spreading away from the original locus, the deeper staining cytoplasm and the gradual recovery into a compact net again. With a dosage of 800 r the massive dark abundance of osmic reducing substance is not so intense at the time when the cell is beginning to recover as it is when a dosage of 1600 r was given.

The cytology of the tumor cells in reference to the time of mitosis, the appearance of the Golgi and of the mitochondria can be noted in Table I.

It may be noted that there is little difference in the morphological changes that can be detected after this dosage at the 3 milliamperages listed, which represent those within the ordinary range of therapeutic radiation. Possibly the series at 25 MA shows the least effect or perhaps the quickest recovery.

There seems to be a period previous to the recurrence of mitosis in which the mitochondria have a greater susceptibility to stain and that

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[8] Warren, S., *Am. J. Roentgenol.,* 1937, **38**, 899.

TABLE I.

Hr after onset of radiation	15 MA	20 MA	25 MA
2 hr	Typical Golgi net. No mitoses. Mitochondria unchanged.	Typical Golgi net. No mitoses. Mitochondria unchanged.	Typical Golgi net. No mitoses. Mitochondria unchanged.
4 hr	Golgi net shattered. No mitoses. Mitochondria unchanged.	Fragmentary Golgi skeins. No mitoses. Mitochondria unchanged.	Slight change in Golgi net. No mitoses. Mitochondria unchanged but susceptible to stain.
6 hr	Fragmented Golgi covering a wide area. Densely staining area in cell. No mitoses. Mitochondria unchanged, little susceptibility to stain.	Wide, granular, densely staining area in cell but net reorganizing. No mitoses. Mitochondria unchanged, little susceptibility to stain.	Wide, granular, densely staining area in cell but net reorganizing. No mitoses. Mitochondria unchanged, some susceptibility to stain.
9 hr	A compact Golgi net in some cells. Dark staining cytoplasm. No mitoses. Mitochondria unchanged but very susceptible to stain.	A compact Golgi net in some cells. Dark staining cytoplasm. No mitoses. Mitochondria unchanged but very susceptible to stain.	A compact Golgi net in most of the cells. Dark staining cytoplasm. Rarely a mitosis. Mitochondria unchanged but very susceptible to stain and absent in Golgi area.
12 hr	Many cells appear normal. Prophases and metaphase apparent. Golgi net in host cells. Mitochondria unchanged.	Same as in 15 MA.	Same as in 15 and 20 MA.

this period, as compared with our earlier work,[1] varies with the dosage. This also holds true for the appearance of mitoses and for the series of changes in the Golgi net. The significance of this cytoplasmic disorganization and subsequent reorganization in relation to dosage remains for subsequent analysis. It is clear, however, that the Golgi in these experiments seems always to reorganize previous to any change in mitotic activity.

We are indebted to Dr. Joseph H. Marks for the radiation of our animals.

10102 P

Vasoconstrictor Properties in Blood of Hypertensive Dogs.

C. Heymans and J. J. Bouckaert.

From the Department of Pharmacology, University of Ghent, Belgium.

Experimental arterial hypertension has been induced in dogs by means of two different methods. In a series of dogs, the 4 moderator nerves (2 aortic and 2 carotid sinus nerves) were cut: both carotid sinuses were completely excised, one cervical aortic-sympathetic-vagus nerve was cut and out of the other aortic-sympathetic-vagus nerve, the aortic and sympathetic fibers were dissected and cut, leaving only a small bunch of vagal fibers sufficient to avoid the accidents of bilateral cervical vagotomy. This technic induces a permanent high blood pressure up to 320 mm Hg for several months. In another group of dogs, chronic arterial hypertension was induced by the permanent compression of the renal arteries, according to Goldblatt's technic.

In order to test the presence of vasopressor substances in the blood of these dogs suffering from chronic hypertension, the following technic was used: In a normal dog, anesthetized with chloralosane and sensitized to vasopressor substances by means of cocaine injection, the volume of the spleen is registered by means of a very sensitive plethysmograph after one branch of the splenic artery has been ligated and prepared for intrasplenic arterial injections. By means of this method, it is possible to register a decrease in the spleen volume caused by an injection into the splenic circulation of adrenalin in amounts as small as 10^{-5} mg.

The vasopressor activity of the arterial blood (1-20 cc) of hypertensive dogs, obtained by femoral puncture and immediately injected into the splenic circulation of the test dog, was tested by means of this very sensitive preparation. These experiments showed that the blood of dogs rendered hypertensive by section of the moderator nerves, possesses, on an average, a higher vasopressor activity than the blood of normal dogs. This fact may be related to the higher sympathetic tone of these animals and, as a consequence, to an increase of the adrenalin content of their blood.[1] No well defined increased pressor response could be observed with the blood of dogs in which chronic hypertension had been produced by renal ischemia. This observation corresponds to the findings of Prinzmetal, Friedman and Oppenheimer,[2] who could not find increased pressor properties in the extracts of blood from patients with hypertension or from dogs with experimental renal hypertension.

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[1] Heymans, C., Le sinus carotidien et les autres zones vasosensibles reflexogenes, Monographie, Revue Belge des Sciences medicales, Paris, Presses universitaires, Londres, Lewis and Co., 1929; Heymans, C., Bouckaert, J. J., and Regniers, B., Le sinus carotidien, Monographie, Paris, Doin, 1933.

10103

Sulfanilamide and Diaminodiphenylsulfone and Their Diacetyl Derivatives in the Treatment of Experimental Intradermal Streptococcus Infections of Rabbits.

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From the Research Institute of Cutaneous Medicine, Philadelphia.

The therapeutic effectiveness of sulfanilamide in experimental beta hemolytic streptococcus infections has usually been determined by the subcutaneous and oral administration of the compound to mice inoculated intraperitoneally.

Upon administration by either route the compound is rapidly absorbed and eliminated. A portion is conjugated into the acetyl derivatives but its therapeutic effects are unknown. It is commonly stated that in human beings the blood should carry from 10 to 12 mg of free sulfanilamide per 100 cc for therapeutic effectiveness.

As shown by Kolmer and Rule[1] the intradermal (shaven abdominal skin) injection of *virulent* beta hemolytic streptococcus in rabbits produces a large severe local lesion of hyperemia and edema followed by necrosis and abscess formation admirably suited for chemotherapeutic investigations since the influence of chemical agents may be determined clinically as well as, and very importantly, by lesion cultures. Septicemia rarely develops but by the simultaneous injection of streptococcus intravenously, septicemia is produced followed by suppurative arthritis in a large percentage of animals.

As shown by Kolmer, Brown, Rule and Werner[2] sulfanilamide in doses of 0.1 to 0.5 g per kilo orally twice daily (6 hours apart) for 5 days in succession usually produced clinical regression of the local

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[2] Prinzmetal, M., Friedman, B., and Oppenheimer, E. T., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 493.

[1] Kolmer, J. A., and Rule, A. M., *J. Lab. and Clin. Med.*, 1937, **22**, 1097.

[2] Kolmer, J. A., Brown, H., Rule, A. M., and Werner, M. L., *J. Lab. and Clin. Med.*, in press.

TABLE I.

Sulfanilamide and Derivatives in the Treatment of Intradermal Streptococcus Infections of Rabbits.*

Compounds	Dose per kilo,† g	Before Treatment		First Day		Second Day		Third Day		Fourth Day		Fifth Day		Seventh Day		Tenth Day	
		Clin.	Cult.	Clin.	Cult.	Clin.	Cult.	Clin.	Cult.	Clin.	Cult.	Clin.	Cult.	Clin.	Cult.	Clin.	Cult.
Sulfanilamide	0.05	4‡	+	4	+	3	+	3	+	3	+	2	+	abs.	+	abs.	+
	0.1	4	+	4	+	3	+	2	+	2	+	2	+	abs.	+	abs.	+
	0.25	4	+	4	+	2	+	2	+	2	+	abs.	+	abs.	+	abs.	+
	0.4	4	+	3	+	3	+	2	+	2	+	1	+	1	+	abs.	+
	0.5	4	+	3	+	2	+	2	+	2	+	1	+	abs.	+	abs.	+
Acetyl Deriv. Sulfanilamide	0.1	4	+	4	+	4	+	4	+	4	+	abs.	+	abs.	+	abs.	+
	0.25	4	+	4	+	4	+	4	+	4	+	4	+	abs.	+	abs.	+
	0.5	4	+	4	+	4	+	4	+	4	+	3	+	abs.	+	abs.	+
	0.8	4	+	4	+	4	+	4	+	4	+	2	+	D	+		
	1.0	4	+	4	+	4	+	4	+	4	+	3	+	D	+		
4,4′-diaminodiphenylsulfone	0.05	4	+	4	+	4	+	3	+	2	+	2	+	abs.	+	abs.	+
	0.1	4	+	4	+	4	+	3	+	2	+	1	+	abs.	+	abs.	+
	0.25	4	+	4	+	4	+	4	+	2	+	1	+	abs.	+	abs.	+
	0.4	4	+	4	+	2	+	2	+	1	+	1	—	1	—	1	—
	0.5	4	+	4	+	2	+	2	+	1	+	1	+	1	—	1	—
Diacetyl deriv. 4,4′-diamino-diphenylsulfone	0.1	4	+	4	+	4	+	4	+	4	+	4	+	abs.	+	abs.	+
	0.25	4	+	4	+	4	+	4	+	4	+	4	+	abs.	+	abs.	+
	0.5	4	+	4	+	4	+	4	+	4	+	4	+	abs.	+	abs.	+
	0.8	4	+	4	+	4	+	4	+	4	+	3	+	2	+	1	—
	1.0	4	+	4	+	4	+	4	+	3	+	2	+	1	+	1	—
Controls	—	4	+	4	+	4	+	4	+	4	+	4	+	abs.	+	abs.	+
	—	4	+	4	+	4	+	4	+	4	+	abs.	+	abs.	+	abs.	+
	—	4	+	4	+	4	+	4	+	4	+	abs.	+	abs.	+	abs.	+
	—	4	+	4	+	4	+	4	+	4	+	4	+	abs.	+	abs.	+
	—	4	+	4	+	4	+	2	+	2	+	2	+	abs.	+	abs.	+

* Inoculated intradermally with 0.6 cc of 18-hour broth culture (room temperature).

† Divided into two parts and given *orally* twice a day (10 A.M. and 3 P.M.) for 5 days in succession (10 doses). First dose 24 hours after intradermal inoculation.

‡ 4 = severe lesion; 3 = less severe, etc.; + abs. = abscess; D = died (positive heart blood cultures).

skin lesions but complete bactericidal effects (negative lesion cultures) were only occasionally and irregularly produceed.

As shown by Bauer and Rosenthal[3] 4,4′-diaminodiphenylsulfone first reported on by Buttle, Stephenson, Smith and Foster,[4] is approximately 30 times as active against streptococci as sulfanilamide in experimental infections of mice. The diacetyl derivative of this compound, introduced by Fourneau, Trefouel, Nitti and Bovet[5] was found to have a therapeutic index 6 times as high as sulfanilamide.

Twenty-five rabbits were inoculated intradermally (shaven abdominal skin) with 0.6 cc (several punctures) of 18-hour broth culture (room temperature) of a *virulent* strain of beta hemolytic streptococcus. Large local lesions of hyperemia and edema with positive cultures resulted in 24 hours.

Five animals were kept as untreated controls and over a period of 10 days all resulted in the production of abscesses with positive lesion cultures at daily intervals (Table I).

Five were given sulfanilamide orally in dose of 0.05 to 0.5 g per kilo per day (divided into 2 doses at 10 a.m. and 3 p.m.) for 5 days in succession, the first dose being administered 24 hours after intradermal inoculation. Clinical improvement began on the second to fifth days of treatment which was well marked by the seventh day, but all animals developed small abscesses with positive daily lesion cultures over the period of 10 days' observation (Table I).

Five were given 0.1 to 1.0 g of the acetyl derivative of sulfanilamide orally per day (divided into 2 doses at 10 a.m. and 3 p.m.) for 5 days in succession, but with practically no clinical evidences of therapeutic effectiveness; all lesion cultures remained positive throughout the period of 10 days. The rabbits receiving 0.8 and 1.0 g per day died after the fifth day of treatment due to toxicity of the compound (Table I).

Five animals were given 0.05 to 0.5 g 4,4′-diaminodiphenylsulfone per kilo per day (divided into 2 doses at 10 a.m. and 3 p.m.) for 5 days in succession. Clinical improvement began on the second to third days of treatment but rabbits receiving 0.05 to 0.25 g daily continued to show positive lesion cultures over the 10 days of observation. Two receiving 0.4 and 0.5 g daily showed practically complete healing on the fifth day of treatment with negative lesion cultures (Table I).

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[3] Bauer, H., and Rosenthal, S. M., *Public Health Reports,* 1938, **53**, 40.

[4] Buttle, G. A., Stephenson, D., Smith, S., and Foster, G. E., *Lancet,* 1937, **1**, 1331.

[5] Fourneau, E., Trefouel, J., Nitti, F., and Bovet, D., *Compt. rend. Acad. d. Sci.,* 1937, **204**, 1763; *ibid.,* 1937, **205**, 299.

Five were given the diacetyl derivative of 4,4′-diaminodiphenylsulfone orally in doses of 0.1 to 1.0 g per kilo daily (divided into 2 doses at 10 a.m. and 3 p.m.). Doses of 0.1 to 0.5 g per kilo produced no clinical effects and lesion cultures were positive throughout; doses of 0.8 and 1.0 g per kilo produced some healing on the fourth to fifth days of treatment which resulted in sterile lesion cultures on the tenth day.

Summary. (1) Sulfanilamide by oral administration to rabbits showed curative effects upon local intradermal streptococcus lesions but did not result in complete bactericidal effects in the dosage employed. (2) The acetyl derivative of sulfanilamide was practically without curative effects. (3) 4,4′-diaminodiphenylsulfone was more curative than sulfanilamide and produced complete bactericidal effects in the larger doses. (4) The diacetyl derivative of 4,4′-diaminodiphenylsulfone was without curative effects in the same dosage as sulfanilamide but produced some curative effects in higher dosage. (5) The results with sulfanilamide, 4,4′-diaminodiphenylsulfone and its diacetyl derivative by oral administration to rabbits with experimental intradermal streptococcus infections have not been as good as reported in the treatment of experimental streptococcus infections of mice by parenteral administration; the differences in results may be due to the difference in the species of animals employed and partly to the routes of administration.

10104

Ineffectiveness of Sulfanilamide in the Treatment of Canine Filariasis.

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Recent reports indicate that sulfanilamide is ineffective in the treatment of several animal parasites.[1, 2] However, as helminths vary so greatly in habitat, morphology and physiology, it appears possible that sulfanilamide may have a selective action upon certain species just as it appears to have on the coccal group of bacteria.

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* I wish to acknowledge the assistance of four medical students, R. D. Baxley, A. K. McAnally, E. Spicer, and J. L. Wardlaw.

[1] McCoy, O. R., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 461.

[2] Keil, E., *Arch. f. Schiffs. u. Trop. Hyg.*, 1936, **40**, 400.

Further, as the treatment of a number of helminthic infections is unsatisfactory, any therapeutic agent which may aid is welcome. Canine filariasis (heartworm) due to *Dirofilaria immitis* is a helminth infection which is very difficult and sometimes impossible to cure with the drugs now used. It was therefore thought worth while to test the value of sulfanilamide in *Dirofilariasis.*

Four infected dogs were treated with varying amounts of sulfanilamide† (para-amino-benzene-sulfonamide). The drug was administered orally for periods ranging from 15 to 36 days. It was given as a single daily dose. The dosage of sulfanilamide varied from 0.057 to 0.137 g per kilo weight of dog. These amounts were all well tolerated, no evidence of toxicity manifested, and all the dogs gained weight during the experiment. Microfilaria counts on 0.1 cc amounts of blood from the infected dogs were made before, during, and after treatment. In order to avoid variations due to periodicity of the parasite, blood for these counts was all secured at the same hour of the day.

In general it appears that the course of sulfanilamide treatment was without significant effect on the microfilaria content of the blood, Table I. The microfilaria counts of dogs 1, 3, and 4 remained rather constant during and for as long as 10 days after the

TABLE I.
Sulfanilamide Treatment of Dogs Infected with *Dirofilaria immitis.*

Dog	No. days of treatment	Daily dose of drug, g	Total dose, g	Microfilariæ per cc of blood	
1	9	0.97	61.1	Before treatment	210
				9th day of ”	190
17 kg	27	1.94		18th ” ” ”	260
				26th ” ” ”	340
				36th ” ” ”	250
				7th ” after ”	230
2	15	2.92	43.8	Before treatment	415
				9th day of ”	260
21.8 kg				15th ” ” ”	210
				10th ” after ”	230
3	15	2.92	43.8	Before treatment	90
				9th day of ”	55
21.0 kg				15th ” ” ”	110
				6th ” after ”	100
				10th ” ” ”	60
4	15	2.92	43.8	Before treatment	2500
				6th day after ”	2200
21.8 kg				10th ” ” ”	2610

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† The drugs used in this study were kindly supplied by the Winthrop Chemical Company.

conclusion of the treatment. In the case of Dog 2, however, the microfilaria count fell approximately 45% after 9 days of treatment. Subsequent counts during the continuation of the treatment and 10 days post treatment confirmed this larval reduction. It is possible that this apparent reduction in microfilaria was not due to the drug but to normal physiological variations. That such variations may occur is indicated by the microfilaria counts of Dog 1, which increased during treatment with Prontosil.

A week after the conclusion of the sulfanilamide therapy by mouth, Dog 1 was given a series of intramuscular injections of a soluble sulfonamide. This treatment consisted of 2 daily intramuscular injections of 10 cc of a 2.5% solution of disodium 4-sulfamidophenyl-2-azo-7-acetyl-amino-l-hydroxynaphthalene 3,6-disulphonate (Prontosil, Winthrop). The drug was administered over a period of 9 days. The microfilaria count which was 250 per cc before treatment rose to 420 on its completion.

It is apparent from the results obtained that sulfanilamide in the amounts given and over the time continued is without effect on *Dirofilaria immitis* as judged by microfilaria counts.

10105 P

Ocular Rotation. Influence of Anesthetics and Operations on Various Parts of the Central Nervous System.

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In experiments on the brainstem of cats the appearance of a striking oblique position of the pupils was observed, indicating an asymmetrical inward rotation of the eyeballs. The question presented itself as to how far this rotation was due to the anesthetics used. In experiments on 67 cats before and during the course of anesthesia the position of the pupil or of a linear scar burnt into the anesthetized cornea as closely as possible to the vertical diameter, was recorded. The smallest range of rotation (8½° outward to 7½° inward) was noticed under the influence of ether, chloroform, and Gréhant's mixture (5% chloroform in 50% alcohol). A variety of effects could be observed: in or outward rotation of one eye only or of both eyes, but also rotation of both eyes in the same direction ("homonymous rotation"). The effect of barbiturates (sodium barbital, sodium phenobarbital, sodium pentobarbital, dial-urethane) and also of chloralose and urethane was much more marked, the range of rotation reaching from 35° inward to 30° outward. In the majority of the cases of this second group the eyes were inwardly rotated (bilateral inward rotation in 31 of 49 observations, inward rotation of only one eye in 4 cases). Much more rarely outward rotation appeared (4 instances of bilateral outward rotation of relatively slight degree), or rotation of both eyeballs in the same direction (10 observations). The tendency for homonymous rotation was manifested particularly in dial-urethane anesthesia (3 of 6 cases). The rotation is usually more marked in one eye than in the other and subject to variations during the anesthesia; often, however, the eyeballs retain the same degree of rotation for several hours. Involuntary horizontal and more rarely vertical movements of the eyeballs appeared in some experiments, but the rotation was as a rule independent of these motor phenomena.

Thus particularly those anesthetics that act mainly on the brainstem, such as the barbiturates, produce a marked ocular rotation, while cortex-anesthetics, such as ether, chloroform, or chloroform-alcohol, have only a slight effect. A sharp division between cortex- and brainstem anesthetics seems, however, not possible, since a so-called cortex-anesthetic (chloralose) may produce a marked bilateral inward rotation.

For further analysis of these phenomena the influence of various parts of the central nervous system upon the eyeballs with special reference to rotation around their antero-posterior axis was studied (stimulation and extirpation experiments under superficial ether anesthesia).

Stimulation of the cortical oculogyric centers in the frontal or occipital lobe produces, besides conjugate deviation to the opposite side and less frequently vertical deviation, a slight rotation in the majority of the experiments. The most frequent reaction is the rotation of the opposite eye towards the stimulated hemisphere (in regard to the upper end of the vertical diameter of the cornea, average less than 5°, maximum 15°). The reaction of the homolateral eye is more variable. The inward rotation of the opposite eye may be accompanied by a slighter inward rotation of the homolateral eye, or the homolateral eye may rotate outwardly, or a reaction of the ipsilateral eye may be absent. In a few instances outward rotation was observed in the homolateral eye only, or this reaction was accompanied by a weaker outward rotation of the opposite eye. Extirpation of the frontal or occipital oculogyric centers or combined elimination of these areas resulted only in a slight and temporary inward rotation reaching not more than a few degrees. Thus a tonic effect of the cortex upon the position of the eyes in regard to rotation could not be found. The inward rotation of the eyeballs became somewhat more pronounced (at an average of 3°) when the decortication was followed by elimination of the subcortical ganglia in front of the mesencephalon. Such high degrees of rotation as were obtained under the influence of anesthetics were, however, not reached. This may be partly due to impairment of the tone of the mesencephalic ocular nuclei after section in front of the midbrain. Furthermore, a direct action of the anesthetics upon the lower parts of the brainstem, reaching the rhombencephalic vestibular nuclei must be considered. This is suggested by cases with marked homonymous ocular rotation, which is usually of unequal degree in both eyes and which reaches much higher degrees (up to 25°) than observed in the above-mentioned cortical experiments. Such marked homonymous rotation appears after unilateral labyrinth extirpation (Rademaker[1]). In our labyrinthectomies this rotation was more developed in the opposite eye (average 20°) than in the homolateral eye (average 7°); it could still be observed after the nystagmus following the extirpation subsided. Thus the fact that the homonymous rotation in anesthesia may appear without nystagmus does not speak against the assumption that the effect of the anesthetic in these cases reaches the rhombencephalic vestibular nuclei disturbing the balance between the nuclei of both sides.

Summary. Under the influence of anesthetics, particularly of the so-called brainstem anesthetics, a marked ocular rotation was observed. The analysis of this phenomenon by experiments on cortex, brainstem, and labyrinth, indicates that it is due not only to the elimination of pros- and diencephalic impulses but in part also to an effect upon lower parts of the brainstem.

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[1] Rademaker, G. G. I., Réactions labyrinthiques et Equilibre, Masson, Paris, 1935.

10106 P

Rate of Inactivation of Equine Encephalomyelitis Virus (Eastern Strain) Relative to H ion Concentration.*

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Detailed studies of the pH stability range of infectivity of plant viruses, summarized recently by Stanley,[1] show that for each virus studied there is a well defined region where infectivity is relatively stable. On both sides of this region infectivity is lost in a regular way so that the curve plotted to relate infectivity with pH of the medium is smooth and trapezoidal. Certain of the animal viruses[2, 3] appear to behave in a like manner. It was entirely unexpected, then, when conditions were encountered under which the virus of equine encephalomyelitis (Eastern Strain) seemed to behave in a different way.

The experiments were made on virus propagated in chick embryos.[4] Virus-infected embryos were "harvested" when moribund and ground with sand in hormone broth to a 10% suspension. Centrifuged free of sand and gross tissue particles, the whole extract was mixed with composite buffer solution (0.05M) of various pH's.[2, 3] Mixtures were maintained between 0°C and 5°C, and after various intervals, usually 1 hour, 1 day, and 1 week, portions were removed for test. The pH of each was readjusted approximately to neutrality and virus infectivity determined by titrations in decimal dilutions in mice.[5] pH was determined and frequently checked with the glass electrode.

Four such experiments covering the range from pH 1.0 to pH 12.0 have shown essentially uniform results, and one is summarized in Fig. 1. The curve after 1 hour is similar in contour to those of other viruses, the region of greatest stability lying between pH 3.5 and pH 11.5. After 1 week, maximum stability is at pH 7.5 to

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* This work was made possible through the interest and aid of Lederle Laboratories, Pearl River, N. Y.

[1] Stanley, W. M., Harvey Lectures, 1937-1938.

[2] Beard, J. W., and Wyckoff, R. W. G., *J. Biol. Chem.*, 1938, **123**, 461.

[3] Beard, J. W., Finkelstein, H., and Wyckoff, R. W. G., *Science*, 1937, **86**, 331.

[4] Beard, J. W., Finkelstein, H., Sealy, W. C., and Wyckoff, R. W. G., *Science*, 1938, **87**, 490.

[5] Olitsky, P. K., and Cox, H. R., *J. Exp. Med.*, 1936, **63**, 311.

pH 8.5, while a second region of relative stability is apparent between pH 3.5 to pH 5.0. About midway between pH 5.0 and pH 6.5 inactivation is almost complete. The curve after 1 day is similar in shape, though the inverted peak between pH 5.0 and pH 6.5 is not so prominent as that after 1 week.

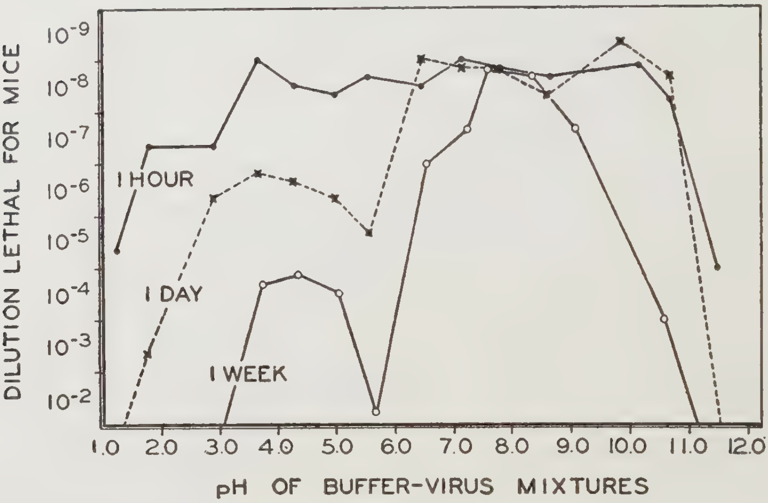

Fig. 1.

pH-activity curves for unpurified equine encephalomyelitis virus (Eastern Strain) in crude extract of chick embryo tissue in which virus was propagated.

The behavior of the virus in the limited region of rapid inactivation was examined in more detail by increasing the number of mixtures with smaller differences in pH between pH 3.0 and pH 6.5. It was found that, instead of a single restricted point, there was a narrow inverted plateau bounded by pH 5.1 and pH 5.7 where inactivation seemed at a maximum.

The possibility existed that this phenomenon might be associated with changes in the virus at its iso-electric point. The iso-electric point of the virus purified by ultracentrifugation,[6] however, determined with the Northrop-Kunitz microcataphoresis cell, was found to lie between pH 3.7 and pH 4.1, far below the region of rapid inactivation. This result was corroborated with the Todd macrocataphoresis apparatus. The purified, fully infectious virus still possessed a negative charge at pH 4.5.

An obvious factor to be considered is the presence of a substance, possibly of the nature of an enzyme,[7] in the chick embryo tissue capable of inactivating the virus under optimum conditions of pH. Preliminary experiments with purified virus indicate that this may indeed be the case. With the purified virus free of chick tissue extract and hormone broth the region of rapid inactivation between pH 5.0 and pH 6.5 has not been apparent. Further experiments in which no hormone broth was employed have indicated that the inactivating material may be present also in normal chick embryo tissue. These possibilities are being investigated further.

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[6] Wyckoff, R. W. G., Proc. Soc. Exp. Biol. and Med., 1937, **36**, 771. The instrument employed was the Wyckoff quantity type and we are grateful to Mr. W. Hurst of the Department of Physics for his interest in its construction and maintenance.

10107

An Antidiuretic Effect Produced by Certain Preparations of Heparin.*

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In attempts to get evidence of the presence of an antidiuretic hormone in blood[1] it was found that commercial preparations of heparin produce antidiuresis when injected intravenously into diuretic rabbits in doses of 1 to 4 mg. Six preparations have been used in 62 injections. Three of 4 lots, obtained from the Connaught laboratories and derived from beef lung, contained 15 anticoagulant units per mg; the fourth, a highly purified solution (lot 43-1), obtained through the courtesy of Dr. C. H. Best, contained 1000 units per cc. Of 2 lots obtained from Hynson, Westcott and Dunning, both derived from dog liver, one contained 5 units per mg, the other 20 units per mg. All except lot 43-1 consistently produced brief definite antidiuresis (Fig. 1), characteristically preceded by a 10-minute latent period, when injected intravenously into rabbits. No effect was produced by subcutaneous injection into rats or frogs.

The antidiuretic action is not a property of the anticoagulant factor in the heparin, for the most potent Connaught specimen had little or no antidiuretic action and the effects of the 2 Hynson, Westcott and Dunning preparations were not proportional to their anticoagulant potencies.

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[7] Best, R. J., and Samuel, G., *Ann. Appl. Biol.*, 1936, **23**, 759.

* The expenses of this work were defrayed in large part from a grant by the Commonwealth Fund.

[1] Walker, A. M., *Am. J. Physiol.*, 1938, **123**, 210.

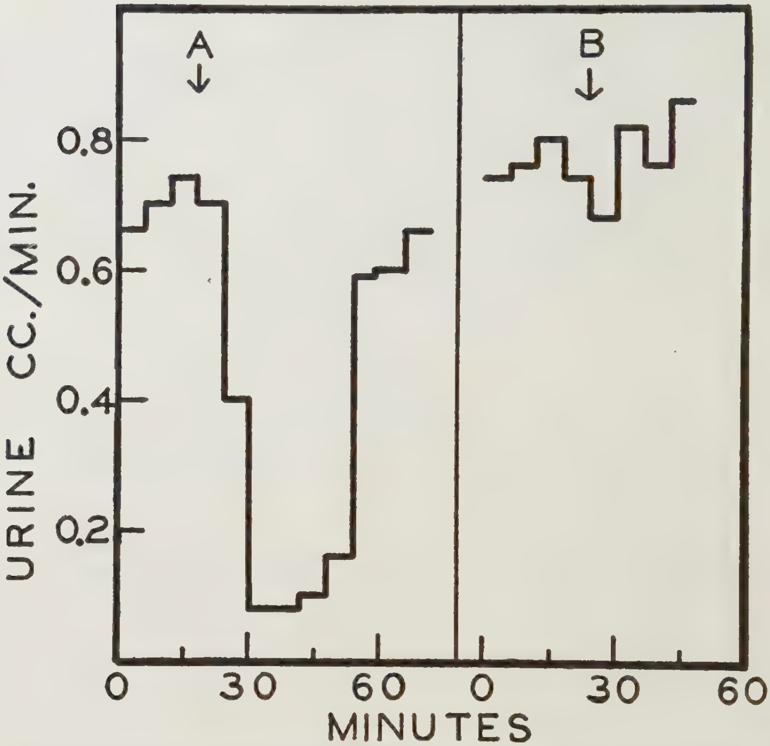

FIG. 1.

Typical record of the urine rate of a diuretic rabbit following the intravenous injection of 2.0 cc 0.9% sodium chloride solution containing (A): 30 units (2 mg) of heparin, Connaught Laboratories, lot 8-5; (B): 100 units (0.1 cc) of purified heparin, Connaught Laboratories, lot 43-1.

It is entirely problematical whether this finding has any relation to the antidiuretic activity of liver extracts described by Theobald and White[2] or to a possible influence of the liver upon kidney function such as is intimated by the chronic polyuria which develops in Eck-fistula dogs.[3, 4] There is no previous record of antidiuretic activity in lung extracts. The purpose of this note is to call the attention of those interested in antidiuretic hormones to the error which the use of heparin entails.

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[2] Theobald, G. W., and White, M., *J. Physiol.*, 1933, **78**, 18P.

[3] Crandall, L. A., Jr., and Roberts, G. M., *Am. J. Physiol.*, 1936, **117**, 318.

[4] Knutti, R. E., Erickson, C. C., Madden, S. C., Rekers, P. E., and Whipple, G. H., *J. Exp. Med.*, 1937, **65**, 455.

10108 P

Abnormalities of the Urinary Tract in "Essential Hypertension."

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This communication is the first of a series in which will be described the situation and the course of events in a group of patients suffering from so-called "essential hypertension." Because of recent interest in the relation of the structure and function of the kidneys to arterial hypertension, both experimental[1] and clinical[2, 3, 4] special studies by a number of methods are being made of the kidneys of patients in whom the diagnosis of "essential hypertension" has been made. One of the methods utilized involves taking X-ray photographs of the kidneys after the intravenous injection of diodrast (3.5 diiodo-4-pyridon-N-acetic acid diethanolamine). This report deals with the results of a study by this method of 71 patients.

The ingestion of fluids was restricted for a period of 36 hours. The night before the examination a mild cathartic was administered. Twenty cc of diodrast was slowly injected intravenously, and X-ray photographs made of the abdomen 5 minutes and 15 minutes after the end of the injection. The patient was then instructed to drink water until urine was passed, and photographs were taken periodically until the renal pelves appeared clear of the injected substance.

The cases of "essential hypertension" were selected for the most part only in that they were relatively young, and that diminution in renal function as measured by the ability of the kidneys to concentrate urine and the standard urea clearance (Van Slyke) had not occurred. The oldest patient was 62, the youngest, 18. The average age at onset of hypertension was 29. An occasional patient was selected because of a history suggestive of renal disease.

Of these 71 patients, some abnormality of the kidneys or ureters was demonstrable in 50. The nature of these abnormalities was as follows:

1. Lesions suggesting obstruction to the flow of urine were found in 36 patients. There was well-marked bilateral hydronephrosis in 4, moderate in 7; unilateral hydronephrosis was present in 20, being

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[1] Goldblatt, H., Lynch, J., Hanzal, R. F., and Summerville, W. W., *J. Exp. Med.*, 1934, **59**, 347.

[2] Leiter, L., *J. Am. Med. Assn.*, 1938, **111**, 507.

[3] Longcope, W. T., *Ann. Int. Med.*, 1937, **11**, 149.

[4] Butler, A. M., *J. Clin. Inv.*, 1937, **16**, 889.

moderate in 11, mild in 9. In 4 of these it was associated with diseases of the other kidney, as follows: (a) marked contraction and distortion of the renal pelvis possibly of inflammatory origin; (b) marked ptosis; (c) total absence of function; and (d) replacement of the kidney by calcified cysts. The obstructions seemed all to be due to constriction of the ureter at the uretero-pelvic junction. In 5 additional patients hydroureter was observed. In one of these marked ptosis of one kidney was present, the other kidney being small and functioning poorly; in a second, a double kidney and mild unilateral hydronephrosis was seen.

2. Renal calculi were seen in the pelves of the kidneys or in their calyces in 5 patients. In one there was also hydronephrosis, and in another, total absence of function in the other kidney.

3. Miscellaneous renal lesions: (a) In 4 patients there was no excretion of diodrast by one kidney on repeated injections, suggesting some interference with renal function on that side; (b) in 2 there was ptosis of one kidney; (c) there was also one case of double kidneys and double ureters; (d) one showing marked distortion of the calyces, possibly a result of old inflammation, and (e) one exhibiting a persistent sharp angulation of the ureter.

Intravenous pyelograms were normal in the remaining 21 patients.

Summary. Abnormalities of the urinary tract, as demonstrated in pyelograms obtained after intravenous injection of diodrast are frequently present in young persons suffering from "essential hypertension." In 50 of 71 patients some abnormality was present. Under these circumstances it seems desirable to explore further the meaning of these phenomena.

10109 P

A New Factor Required for Growth and Reproduction in the Domestic Fowl.

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In an attempt to determine whether or not the chick antidermatosis vitamin (filtrate factor) is required for reproduction in the domestic fowl a basal diet was fed composed of 40.65% yellow corn meal, 20% wheat bran, 20% wheat flour middlings, 7% purified casein, 5% soybean oil, 0.5% wheat germ oil, 0.5% cod liver oil, 0.35% liver extract, 3.5% pulverized limestone, 2.0% steamed bone meal, 0.5% salt and a trace of manganese carbonate. The antidermatosis vitamin in the cereal products and the liver extract was destroyed by heating in an oven for 36 hours at 120°C[1] while the casein was freed of the vitamin first by washing repeatedly at pH 2.5 in the presence of sufficient salt to prevent solution of the casein and then by washing at pH 4.7, the isoelectric point of casein.[‡] By means of appropriate chemical and biological procedures the heated diet was demonstrated to be adequate in all known vitamins except the antidermatosis vitamin and nicotinic acid.

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* Anheuser-Busch Fellow.

† Borden Investigator.

When the heated diet was fed to hens the percentage hatch of the fertile eggs gradually decreased. The percentage hatch of the fertile eggs of the hens fed the unheated diet, on the other hand, increased somewhat. The results of this work are presented in Table I.

TABLE I.
Effect of Heated Diet upon Reproduction.
(80 hens at start.)

	No. eggs set	No. hatches	% hatch of fertile eggs
Preliminary period			
Unheated diet	666	2	53.1
Depletion period			
Unheated diet	560	6	63.2
Heated ”	1076	6	13.9
Experimental period			
Unheated diet	536	7	67.4
Heated ” (HD)	328	7	4.5
HD + nicotinic acid	281	7	4.3
HD + antiderm. vit. conc.	370	7	13.2

When the hatchability of the eggs of the hens fed the heated diet reached its lowest point, the hens were divided into 3 groups. One of these groups continued to serve as a control, one was fed nicotinic acid and one was fed a concentrate of the antidermatosis vitamin which had been biologically assayed and found to contain about 25 units of the vitamin per gram.[2] This concentrate also contained nicotinic acid. No improvement in hatchability was obtained by feeding nicotinic acid at levels of 1.5 mg and 3 mg per hen per day, amounts in the upper limits of the range reported to prevent black tongue.[3] A slight improvement was obtained by feeding the vitamin concentrate at a level of 5%. No further improvement, however, was obtained by increasing the vitamin concentrate to 10%, a level providing 2.5 times as much of the antidermatosis vitamin as is needed to prevent dermatosis in chicks.[2]

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[1] Keenan, J. A., Kline, O. L., Elvehjem, C. A., and Hart, E. B., *J. Nutr.*, 1935, **9**, 63.

[‡] Unpublished method of Sharpe, P. F., and de Tomasi, J. A., Cornell University.

[2] Jukes, T. H., and Lepkovsky, Samuel, *J. Biol. Chem.*, 1936, **114**, 117.

When chicks were fed a heated diet supplemented with the concentrate of the antidermatosis vitamin lesions of dermatosis did not develop but the rate of growth was significantly less than that obtained on the unheated diet even when twice the amount of concentrate required to prevent dermatosis was fed.[2] The results of this study are presented in Table II.

TABLE II.
Effect of Heated Diet on Growth.
(14 chicks per lot.)

Lot	Treatment	% degree dermatosis	Wt in g at 5 wks.	"t" value of difference between preceding lot*
1	Heated diet (HD)	37.6	89.0	—
2	HD + 1% antidem. vit. conc.	27.4	114.2	2.29
3	HD + 2% " " "	19.7	152.1	3.61
4	HD + 4% " " "	2.8	184.6	2.25
5	HD + 8% " " "	0.0	206.0	2.00
6	Unheated diet	0.0	266.5	5.44

*t = 1.99, odds 19:1; t = 2.64, odds 99:1.

The factor has been found in yeast and in milk as well as in cereals. In the bioassays showing this chicks were fed a diet complete in all known nutritive essentials except riboflavin. When yeast and milk were fed so as to provide 100γ of riboflavin per 100 g of diet the rate of growth in a number of experiments was significantly greater than that of comparable chicks fed the basal diet supplemented with 100γ of crystalline riboflavin per 100 g of diet. The rate of growth was also greater than that of comparable chicks fed 250γ of riboflavin per 100 g of diet, a level of riboflavin together with that in the basal diet sufficient to promote optimum growth, if the diet had been otherwise complete.

It is improbable that any amino acid deficiency exists in the heated diets, since a diet similar to the unheated diet used in demonstrating the presence of the new factor in yeast and milk has been found in protein balance experiments[4] to promote approximately as great a protein retention in growing chicks as any of the other numerous protein combinations studied. It has also been shown that by supplementing heat-treated cereals with casein the biological value of the combination is equal to that of the unheated cereals plus casein.[5]

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[3] Elvehjem, C. A., Madden, R. J., Strong, F. M., and Woolly, D. W., *J. Biol. Chem.*, 1938, **123**, 137.

[4] Wilgus, H. S., Jr., Norris, L. C., and Heuser, G. F., *J. Agr. Res.*, 1935, **51**, 383.

Conclusions. It is evident from these results and also from the known character of the ration fed that a factor is required for growth and reproduction in the domestic fowl other than vitamins A, B, B_4, B_6, D, E, K, riboflavin, the chick antidermatosis vitamin, nicotinic acid, factor W, the antiencephalomalacia factor and the essential fatty acids. It appears improbable that this factor is the grass juice factor,[6] since corn and wheat are reported to be poor sources of the latter factor. It also is improbable that the factor is protein in nature. The factor is destroyed by heating in a dry atmosphere for 36 hours at 120°C. It is present in cereals, yeast and milk.

10110 P

Development of the Mesonephros in Amblystoma after Early Extirpation of the Duct.

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The mesonephros in Amblystoma develops from the 9th (or 10th) embryonic somite to the twentieth, at the level of the cloaca. The anterior region, corresponding approximately to segments 10-15, bears the usual relation to the gonad and is the so-called "sexual kidney." The posterior region, more massive and anatomically more complex (due to the presence of secondary, tertiary units, etc.) is the "secretory kidney." Both regions develop from a continuous band of nephrogenic tissue, the "mesonephric blastema" dorso-medial to and contiguous with the primitive kidney duct or pronephric duct. Very early in differentiation the mesonephric units of both regions establish connections with this duct, which thus becomes the mesonephric duct. There is, however, a marked delay in the development of the posterior region, during which the units of the sexual kidney are well developed and functional; and it is to this region of delayed development that secondary, tertiary, etc., tubules are confined. These statements rest largely on the early work of Hall.[1] Such differences in morphology, chronology and functional relations raise interesting problems of homology.

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[5] Morgan, A. G., *J. Biol. Chem.*, 1931, **90**, 771.

[6] Kohler, G. O., Elvehjem, C. A., and Hart, E. B., *Wisc. Agr. Exp. Sta. Bul.* 439, 1937, 2.

In view of the early association between the rudimentary mesonephric units of both regions and the duct, it was decided to investigate the result of elimination of the latter upon the origin and differentiation of the former. In Amblystoma the primitive duct (pronephric duct) arises at the level of somites 3 and 4, and gradually grows posteriorly along the ventral border of the row of somites until it reaches the cloaca at the level of somite 20. At stages 26-27, Harrison's Series, the growing end of the duct reaches only to the 8th or the beginning of the 9th somite, and at or prior to this time may be removed without disturbing the mesonephric region. The blastema is untouched, but will never establish relations with a duct. Embryos subjected unilaterally to this operation were studied at several larval stages, comparing operated and unoperated sides. Earlier results, chiefly on *A. tigrinum,* have been reported;[2] further experiments on *A. punctatum* confirm and enlarge these findings. Thus far, because of a special interest in the gonad, the region of the "sexual kidney" has been studied more fully.

The results, based on a study of 22 cases, may be summarized as follows: (1) Gross dissection shows the duct entirely lacking on the operated side. In about one-third of all cases only a slender, beaded strand of tissue marks the position of the mesonephros. In the remaining cases nodular masses occur irregularly at one or more loci, denoting the development of tubules, singly or in clumps.

(2) Histologically, all units on the operated side are found to be capable of developing to a vesicular form in the absence of the duct. This is short of the stage at which connection to the duct would occur.

(3) About 15% of all units continue development to the stage of simple sigmoid tubules (stage of connection) or beyond. Many approximate normal tubules, but others are atypical or degenerate.

(4) Independently developing units occur at all levels of the kidney. However, when the data for the whole group are plotted according to spinal segments, tubule development occurs most frequently in the middle segments of the sexual region.

(5) Single isolated tubules may develop but there is a tendency to differentiate in clumps of 2 or more, and for rudimentary units adjacent to differentiated tubules to be more advanced in development.

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[1] Hall, R. W., *Bull. Mus. Comp. Zool. Harvard College,* 1904, **45**.

[2] Burns, R. K., Jr., *Anat. Rec.,* 1934, **58** (Suppl., p. 7).

(6) Although the average number of units exhibiting independent differentiation might suggest that only Hall's "units of the first order" possess this capacity, nevertheless the irregular distribution in individual cases, the tendency to occur in aggregations, and complete failure of all units to develop in many cases, do not favor this hypothesis.

(7) Posterior to the sexual region differences between operated and unoperated sides are less extreme, due to the more recent development of these units. The percentage of tubules capable of independent differentiation is, however, about the same as for the "sexual kidney." A study of older larvae will be necessary before final statements can be made regarding this region.

(8) The fundamental distinction between units capable of independent differentiation and those whose development is arrested at the vesicle stage, remains to be determined.

10111

Susceptibility of the "Gopher," *Citellus richardsonii* (Sabine), to *Leptospira icterohemorrhagiæ*.

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Rats *(Rattus norvegicus* and *Rattus rattus)* are responsible for the world-wide dissemination of bubonic plague and Weil's disease (infectious spirochetal jaundice or spirochetosis icterohemorrhagica) and for the presence of plague in the extensive rodent population that is native to the United States. Plague was originally introduced among the native rodents through the association of infected rats from the wharves of San Francisco with members of the ground-squirrel genus *Citellus*. The disease is continuing to spread in the ground-squirrel host. Today, it is present throughout an extensive area and constitutes a public health problem of major importance. Might ground-squirrels play a similar rôle in Weil's disease? An investigation was undertaken to throw light on this point. In the present communication, evidence is summarized which shows that the "gopher," *Citellus richardsonii* (Sabine), a rodent closely related to the California ground-squirrel, is susceptible to infection by the etiological agent of Weil's disease, *Leptospira icterohemorrhagiæ*.

The Richardson ground-squirrel, *Citellus richardsonii* (Sabine), is representative of a large number of closely related species which are distributed throughout much of the United States and Canada. In the north central states, and spreading over into Alberta and Saskatchewan, an area in which it is the commonest wild animal, it is known by the local name of "gopher". The adult animal, grayish-brown in color, measures 7 to 9 inches over-all. It is characterized, as are the related species, by the possession of long claws and powerful limbs with which it burrows into the ground. One litter of from 6 to 11 offspring is born during the summer months. This explains the great increase in numbers as well as the marked activity during this period.

The natural habitat of the gopher is pastures and fields, where grain, roots, and other vegetation provide an ample food supply. Not infrequently, however, it lives in close proximity to buildings, such as barns and granaries, which are infested with rats. Thus, the rats are able to invade the burrows of the gophers and to manifest their predatory habits. It is significant, also, that gophers are sufficiently carnivorous to kill and devour sick and weakened members of their own or related species. This characteristic promotes the transmission of disease from animal to animal and from species to species.

Two strains of *Leptospira icterohemorrhagiæ* were employed in the present studies. One of these strains (the K. strain, which was isolated from a human case) had been carried through 32 passages in guinea pigs before being used to initiate a series in gophers. The other strain, Rat No. 45, was isolated from rats captured at Rochester, New York.

Blood was used as the infective medium for the initial infection of the gophers with each of the strains of *Leptospira icterohemorrhagiæ*. The rat strain was carried through 5 successive passages in gophers, passage being effected by the intraäbdominal injection of 1 cc of blood. The human strain was carried through 5 passages in gophers, using blood as the inoculum. Then, for an additional 10 passages, the strain was maintained in gophers by the subcutaneous inoculation of 1 cc of a 10% kidney-suspension from which the larger particles had settled out on standing. The kidney-suspension was prepared by trituration (without an abrasive) with 0.85% NaCl solution.

In the gophers of both passage-series, the experimental disease was uniformly fatal. The period of survival varied from 4 to 9 days. The clinical manifestations of the infection in gophers were as severe, if not more so, as in guinea pigs. The pathology was essentially similar, although jaundice was not as readily discernible in the gopher as in young white guinea pigs.

Successful passage of the disease was also effected when gophers, dying or dead of the spirochetal infection, were permitted to remain in a cage with normal gophers. Under these circumstances, the normal animals ate portions of the infected animals, thereby contracting a fatal infection.

No change in the virulence of the organisms was revealed on testing in guinea pigs, after 15 serial passages in gophers.

Summary. Evidence is presented of the susceptibility of the gopher or Richardson ground-squirrel, *Citellus richardsonii* (Sabine), to the etiological agent of Weil's disease, *Leptospira icterohemorrhagiæ*. Two series of 5 and 15 passages through gophers, respectively, were effected by the parenteral introduction of blood or tissue-suspensions. The organisms were tested in guinea pigs following the serial passages in gophers; no changes in pathogenicity were noted. Passage was also accomplished by permitting normal gophers to feed on infected gophers, dying or dead of the spirochetal disease.

The findings presented in this note suggest that gophers, or similar wild rodents, might act in nature as hosts for *Leptospira icterohemorrhagiæ*.

10112

Rate of the Flow of Bile. Experimental Data on the Rabbit.

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Incidental to investigations on the function of the gallbladder the writer and his coworkers[1-4] accumulated data relative to the amount of bile produced by the rabbit. Information obtained under similar

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[1] Halpert, B., and Hanke, M. T., *Am. J. Physiol.*, 1929, **88**, 351.

[2] Halpert, B., and Hanke, M. T., *Am. J. Physiol.*, 1932, **100**, 433.

[3] Halpert, B., Thompson, W. R., and Marting, F. L., *Am. J. Physiol.*, 1935, **111**, 31.

[4] Halpert, B., O'Connor, P. A., and Thompson, W. R., *Am. J. Physiol.*, 1935, **112**, 383.

experimental conditions as to the quantity of bile produced per hour per kilo of body weight in a large number of rabbits has not previously been reported. For this reason, the accumulated data are presented here in some detail.

Method. In each experiment the animal was anesthetized with ether and a glass cannula was tied into the common bile duct close to its termination in the duodenum. In the first series of experiments, a short rubber tube connected the glass cannula with a graduated pipette of 1 cc capacity and the bile was collected in 1 cc quantities. In the second series, the cannula was joined with a rubber tube to the inlet of a glass trap and the bile was collected over successive intervals of one-half hour. In the third series, the bile was similarly obtained but was collected against a hydrostatic pressure of 50, 75, and 100 mm respectively, greater than that of the atmosphere. The duration of the collections was from 2 to 6 hours.

TABLE I.
Volume of Bile Obtained from Ductus Choledochus of the Rabbit.

Rabbit No.	Kg		1st hr ml	2nd hr ml	per kg ml/hr	3rd hr ml	4th hr ml	per kg ml/hr	5th hr ml	6th hr ml	per kg ml/hr
I-4	2.9	—	10	6	2.76	—	—	—	—	—	—
I-8	2.3	♂	9	4	2.82	—	—	—	—	—	—
I-46	2.5	—	7	5	2.40	—	—	—	—	—	—
M-17	2.8	♂	7	5	2.14	—	—	—	—	—	—
I-28	3.9	—	16	18	4.35	17	·	·	—	—	—
I-47	2.1	—	12	10	5.24	7	-	-	—	—	—
I-48	2.2	—	11	11	5.00	8	—	- -	—	—	—
I-30	2.7	—	5	5	1.85	10	—	—	—	—	—
I-6	2.8	♂	10	7	3.03	6	5	1.96	—	—	—
I-7	2.5	—	10	8	3.60	6	5	2.20	—	—	—
M-15	2.4	♂	5	3	1.66	3	3	1.25	—	—	—
I-20	2.4	—	10	10	4.16	9	11	4.16	9	—	—
I-17	3.7	—	10	9	2.56	10	10	2.70	10	—	—
I-25	2.3	♀	17	12	6.30	12	13	5.43	14	—	—
M-4	2.5	♂	9	7	3.20	6	4	2.00	5	—	—
M-20	3.2	♂	5	4	1.40	4	2.5	1.01	2.5	—	—
I-29	2.9	♂	3	3	1.03	3	2	0.86	2	—	—
I-14	3.4	—	13	17	4.41	12	11	3.38	9	8.	2.50
I-23	3.1	—	10	8	2.90	6	8	2.25	5	6.5	1.85
I-32	2.8	♀	10	9	3.39	8	5	2.32	4	3.	1.25
I-31	2.5	♀	9	6	3.00	6	6	2.40	8	8.	3.20
I-34	2.6	♂	9	4	2.50	5	3	1.53	2.5	2.5	0.96
I-16	2.6	—	6	4	1.92	6	6	2.30	6	6.	2.30
I-5	2.6	♂	8	7	2.88	9	7	3.07	9	9.	3.46
M-18	2.6	♂	6	3.5	1.82	3.5	3.5	1.34	4.5	4.	1.63
I-33	2.5	♂	5.5	3.5	1.80	3.5	3	1.30	3	3.5	1.30
I-1	2.5	♂	5.5	6.5	2.40	7	7	2.80	7.5	7.	2.90
I-2	3.1	♂	15.5	13.5	4.67	8	8.5	2.66	9.5	9.5	3.06
I-3	2.5	♀	8.5	6.5	3.00	7.5	6.5	2.80	4.5	5.5	2.00
Mean			9.0	7.4	3.04	7.3	6.2	2.29	6.4	6.0	2.20
a. d.			2.5	2.9	0.96	2.4	2.5	0.76	2.7	1.9	0.70

Experimental Data. In the first series of experiments (Table I), the mean flow of bile per hour per kilo of body weight was 3.04 cc with an average deviation of 0.96 during the first 2 hours (29 rabbits), 2.29 cc with an average deviation of 0.76 during the second 2 hours (21 of the 29 rabbits), and 2.20 cc with an average deviation of 0.70 in the third 2 hours (12 of the 29 rabbits).

In the second series of experiments (Table II), the mean flow of bile per hour per kilo of body weight was 4.00 cc with an average deviation of 1.12 during the first 2 hours (18 rabbits), 3.27 cc with an average deviation of 0.67 in the second 2 hours (12 of the 18 rabbits), and 2.64 cc with an average deviation of 0.47 in the third 2 hours (7 of the 18 rabbits).

TABLE II.
Volume of Bile Obtained from Ductus Choledochus of the Rabbit.

Rabbit No.	(Males) Kg	1st hr ml	2nd hr ml	per kg ml/hr	3rd hr ml	4th hr ml	per kg ml/hr	5th hr ml	6th hr ml	per kg ml/hr
L-11	2.9	9.9	6.2	2.77	—	—	—	—	—	—
C-9	2.5	6.0	6.1	2.42	7.5	—	—	—	—	—
C-11	3.1	17.1	18.7	5.77	14.5	—	—	—	—	—
C-12	2.9	11.6	9.6	3.65	10.7	—	—	—	—	—
C-13	2.8	10.5	10.0	3.66	9.9	—	—	—	—	—
C-14	3.2	11.0	8.1	2.98	5.3	—	—	—	—	—
L-5	3.0	14.2	10.3	4.08	8.7	6.6	2.55	—	—	—
L-6	2.3	6.9	6.0	2.80	5.5	5.6	2.41	—	—	—
L-3	2.4	11.4	8.2	4.08	8.5	9.4	3.72	—	—	—
L-8	2.5	7.1	10.9	3.60	10.7	8.7	3.88	—	—	—
L-10	2.8	19.6	12.8	5.78	10.7	9.7	3.64	—	—	—
L-1	2.7	8.9	8.9	3.29	9.5	9.6	3.53	8.1	7.7	2.92
L-2	2.6	12.8	12.6	4.88	10.8	13.7	4.71	9.1	11.8	4.01
L-4	2.5	18.9	18.8	7.54	11.2	8.2	3.88	7.4	5.9	2.66
L-9	2.5	16.8	13.1	5.98	9.7	8.1	3.56	6.8	5.1	2.38
L-12	2.7	8.0	6.7	2.72	7.5	8.0	2.87	7.5	6.5	2.59
S-1	2.5	10.0	9.7	3.94	8.0	7.0	3.00	5.9	6.1	2.40
S-2	2.2	3.7	5.8	2.15	3.8	2.8	1.50	2.3	4.4	1.52
Mean		11.4	10.1	4.00	8.9	8.1	3.27	6.7	6.8	2.64
a. d.		3.5	2.9	1.12	2.0	1.8	0.67	1.5	1.4	0.47

The amount of bile obtained per hour per kilo of body weight in the first series of experiments was somewhat less than in the second. For this difference the method of collecting the bile may be partly responsible, though it is conceivable that the difference is within the usual limits of variation. Pooling the data of the first and second series of experiments, the mean flow of bile per hour per kilo of body weight was 3.4 cc with an average deviation of 1.02 during the first 2 hours (47 rabbits), 2.64 cc with an average deviation of 0.72 during the second 2 hours (33 of the 47 rabbits), and 2.36 cc with an average deviation of 0.61 during the third 2 hours of the experiments (19 of the 47 rabbits).

In the third series of experiments (Table III), in which bile was collected against a hydrostatic pressure greater than that of the atmosphere, the mean flow of bile per hour per kilo of body weight was 2.77 cc with an average deviation of 0.35 during the first 2 hours (13 rabbits) and 1.63 cc with an average deviation of 0.28 during the second 2 hours (7 of the 13 rabbits), when the pressure was 50 mm greater than that of the atmosphere. The mean flow of bile per hour per kilo of body weight during the first 2 hours of the experiment was 4.56 cc with an average deviation of 0.69 (4 rabbits), when the pressure was 75 mm, and 3.07 cc with an average deviation of 1.02 (6 rabbits), when the pressure was 100 mm greater than that of the atmosphere.

TABLE III.
Volume of Bile Obtained from Ductus Choledochus of the Rabbit.
Hydrostatic Pressure 50 mm.

Rabbit (Males) No.	Kg.	1st hr ml	2nd hr ml	per kg ml/hr	3rd hr ml	4th hr ml	per kg ml/hr
M-27	3.0	10.4	7.2	2.93	—	—	—
M-28	3.1	10.7	7.2	2.88	—	—	—
M-30	3.4	14.8	7.6	3.29	—	—	—
M-31	2.8	9.6	8.3	3.19	—	—	—
M-33	3.8	12.4	12.3	3.25	—	—	—
M-36	3.4	11.1	11.4	3.30	—	—	—
M-24	3.9	11.6	9.7	2.73	8.0	7.7	2.01
M-25	3.6	4.5	6.5	1.52	4.2	5.0	1.27
M-26	3.6	10.9	10.0	2.90	8.8	5.0	1.91
M-29	3.0	10.5	4.5	2.50	4.7	4.2	1.48
M-34	3.6	9.9	9.2	2.68	6.6	7.7	1.98
M-37	3.5	10.2	7.8	2.57	5.3	4.3	1.37
M-32	2.9	6.6	7.0	2.34	4.6	3.8	1.44
Mean		10.2	8.4	2.77	6.0	5.4	1.63
a. d.		1.5	1.7	0.35	1.5	1.3	0.28
Hydrostatic Pressure 75 mm.							
C-2	2.6	8.8	13.5	4.28	11.5	—	—
C-4	2.7	11.2	9.7	3.87	8.1	—	—
C-6	3.0	11.7	13.3	4.16	4.7	—	—
C-17	3.0	20.0	15.7	5.95	12.7	—	—
Mean		12.9	13.0	4.56	9.2	—	—
a. d.		3.5	1.7	0.69	2.8	—	—
Hydrostatic Pressure 100 mm.							
M-38	3.0	9.7	5.0	2.45	—	—	—
C-1	2.4	6.7	4.1	2.25	4.5	—	—
C-5	2.9	11.7	8.7	3.52	7.3	—	—
C-7	2.8	11.3	10.0	3.80	8.4	—	—
C-8	3.1	14.8	12.8	4.45	10.4	—	—
C 15	3.0	6.8	5.0	1.96	2.5	—	—
Mean		10.1	7.6	3.07	6.6	—	—
a. d.		2.4	2.9	1.02	2.5	—	—

Summary and Conclusions. From the data presented it is evident that there was considerable variation in the total amount of bile obtained in each animal. The mean flow of bile per hour per kilo of body weight was 3.4 cc in the first 2 hours in 47 rabbits, 2.64 cc in the second 2 hours in 33 of the 47 rabbits, and 2.33 cc during the third 2 hours of the experiments in 19 of the 47 rabbits. Thus there was a gradual decrease in the flow of bile as the experiments proceeded. This decrease was more marked in those experiments in which the bile was collected against a hydrostatic pressure greater than that of the atmosphere. During the entire period of each experiment the gallbladder was resorbing half the volume of its content per hour, *i. e.*, about 0.5 cc per hour per kilo of body weight,[3, 4] hence the amount of bile actually produced per hour by each rabbit was greater than that recorded.

10113

Reducing Substances of the Normal Urine. I. Effects of Insulin Injection.

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This paper embodies a study of the effect of insulin injection upon the excretion of the reducing substances of the normal urine, with special regard to the fermentable fraction. The observations were made on dogs.

There are two articles in the literature pertinent to our subject. Salmon[1] finds that, in man, there is a correlation between the blood sugar level and the normal excretion of reducing substances in the urine. Van Creveld and Van Dam[2] eviscerated frogs, except for kidneys and ureters, cannulated the latter, and perfused through the renal artery a fluid similar to Ringer's solution. To the perfusion were added various concentrations of glucose, and the glucose concentration of the recovered perfusion fluid and of the artificial urine was determined by the micrometric method of Bang. They noted that as the concentration of glucose in the perfusion fluid was increased, there was a corresponding increase in the concentration of glucose in the artificial urine. Where insulin had been injected before the animal was eviscerated, or where insulin was added to the perfusion fluid, there was a profound fall in the concentration of glucose in the urine, and an increase in the concentration of glucose in the fluid recovered from the renal vein. These workers concluded that the presence of insulin in the kidney decreases the passage of glucose into the urine, and ascribed this action to the existence of a "renal attacking point" for insulin.

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[1] Salmon, U. J., Proc. Soc. Exp. Biol. and Med., 1930, **27**, 823.

[2] Van Creveld, S., and Van Dam, E., *Nederland Tijdschr. Geneeskunde*, 1923, **66**, 11, 1498.

While the results of this experiment were dramatic and doubtless important, the mode of attack was such as to involve distinctly unphysiological conditions, notably in the use of an artificial renal environment. Further, the estimations involved the *concentration* of glucose in the urine and in the recovered fluid, rather than the *quantity* of glucose excreted per unit of time. We have found the latter method more suitable for the estimation of these urinary constituents.

We have used normal animals. Our method was first to obtain control figures for reducing substances in the blood and urine of the fasting animal. Having established the control levels, we administered glucose by stomach tube. Then one unit of insulin per kilo was injected. Further quantities of glucose were given at such intervals as to maintain the blood "sugar" above the control level. Urine samples were taken by catheter at hourly intervals and analyzed. Thus we were able to eliminate the hypoglycemic influence in our observations of the renal passage of reducing substances under the influence of insulin. Intravenous insulin injections gave the same results as those given subcutaneously.

Determinations of reducing substances were made by the Folin-Wu method.[3] Before analysis, the urine was treated with Lloyd's aluminum silicate, as recommended by Folin and Berglund.[4] In determining the fermentable fraction of the reducing substances in the urine, we employed the Folin-Berglund fermentation procedure.[5]

In 18 of 20 experiments the total reducing substances showed a sharp drop in the first hour. This effect persisted for 3 hours (Graph 1, Fig 1).

In each of 6 experiments in which we determined the fermentable fraction of the urinary reducing substances this fraction was practically abolished in the first 2 hours and was very much lower than the control level during the third hour (Graph 2, Fig. 1).

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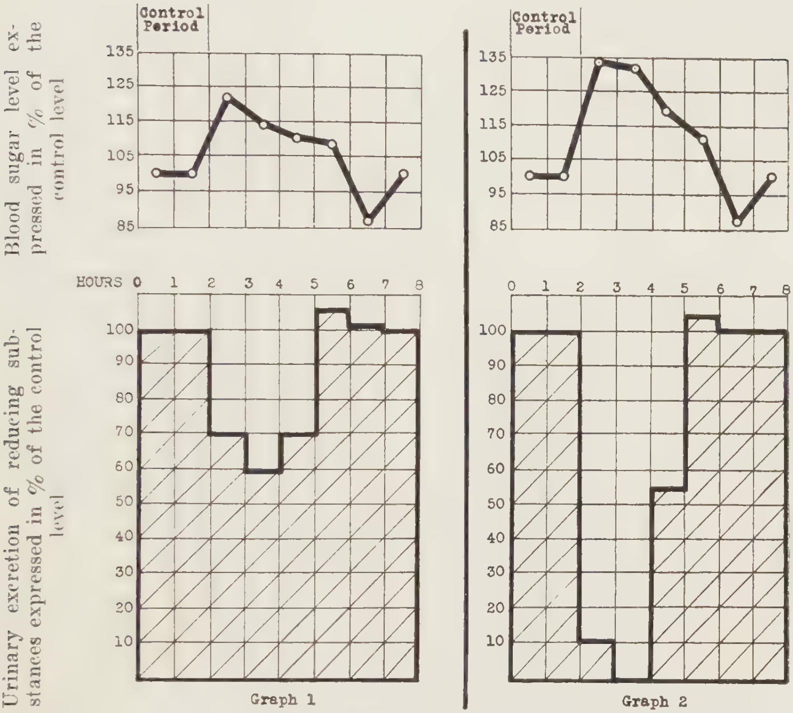
[3] Folin, O., and Wu, H., *J. Biol. Chem.*, 1920, **41**, 367.

[4] Folin, O., and Berglund, H., *J. Biol. Chem.*, 1922, **51**, 209.

[5] VanSlyke, D. D., and Hawkins, J. A., *J. Biol. Chem.*, 1929, **83**, 51.



Graph 1
Total Reducing Substances

Graph 2
Fermentable Reducing Substances

FIG. 1.

The explanatory graphs are arranged as composites of the values obtained from all experimental data, the values being expressed in percentages. The average initial blood sugar was 97 mg per 100 cc. The average initial total reducing substances was 14.4 mg per hour and the average initial fermentable reducing substances was 7.5 mg per hour, in the urine.

The data reported here seem to indicate that insulin acts to effect a diminution in the hourly excretion of fermentable reducing substances in the urine, by some mechanism other than its effect on the blood sugar.

10114 P

Observations upon Allergic Reactions Produced with Various Bacilli of the Acid-Fast Group.

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The two representative pathogenic bacilli of the acid-fast group are *B. tuberculosis* and *B. leprae.*

The phenomena of allergic reactions in tuberculosis have been well established clinically and experimentally. The experimentalists have obtained various forms of such reactions and have arrived at divergent opinions concerning their relationship to aggravation of or protection against the disease. Thus, Dembinski,[1] Rolland,[2] Krause and Willis,[3] and Clawson[4] consider that despite the allergic state present or produced experimentally by inoculation with *B. tuberculosis,* the resistance to subsequent infection is greatly exalted. On the other hand, Selter,[5] Rich, Jenning and Downing[6] regard the sensitized state as a precipitating factor of increased susceptibility. Pagel[7] states that there is no parallelism between tuberculin-supersensitivity and immunity.

In connection with leprosy, the use of leprolin has, in general, been especially applied to this disease. Cummins and Williams[8] carried out some combined tests with tuberculin and leprolin upon human subjects and considered that there existed some group-sensitivity.

It is to be noted, however, that but little, if any, of the results of experimentation has been published relative to presence or absence of specific allergic reactions produced by various members of this extensive group either in regard to infection with *B. tuberculosis* or *B. leprae.*

The work of Harris and Lanford,[9, 10] Lewis and Aronson,[11] and

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[1] Dembinski, B., *Ann. de l'Inst. Past.*, 1899, **13**, 426.

[2] Rolland, J., Etude sur le phenomene de Koch et la reinfection tuberculeuse, Paris, G. Steinheil, 1914.

[3] Krause, A. K., and Willis, H. S., *Tubercle,* London, 1925, **6**, 438.

[4] Clawson, B. J., *Arch. Path.*, 1936, **22**, 99.

[5] Selter, H., *Deut. med. Woch.*, 1925, **51**, 933.

[6] Rich, A. R., Jenning, F. B., and Downing, L. M., *Bull. Johns Hopkins Hosp.*, 1933, **33**, 172.

[7] Pagel, Walter, *J. Path. and Bact.*, 1937, **44**.

[8] Cummins, S. L., and Williams, E. M., *British Med. J.*, 1934, **1**, 702.

[9] Harris, W. H., and Lanford, J. A., *J. Inf. Dis.*, 1913, **13**, 301.

[10] Harris, W. H., and Lanford, J. A., *J. Med. Res.*, 1916, **34**, 157.

[11] Lewis, P. A., and Aronson, J. D., *J. Exp. Med.*, 1923, **38**, 219.

Cooke[12] has shown that serological methods do not afford a satisfactory means of differentiation of various strains or members of the acid-fast group. Such results have been attributed to the supposedly common lipoidal coat affording the acid-resistant factor.

Although the specific identification of most of the members of this extensive group is readily accomplished by pathogenicity, cultural and other tests, the differentiation of certain of these strains is especially important in connection with cultures recovered from leprous lesions.

In the present preliminary work, we have endeavored to ascertain if allergic reactions obtained by employing certain of the acid-fast bacilli were of a group or differential character. The acid-fast species employed were *B. leprae,* chrome (Duval), *B. leprae,* nonchrome (Kedrowski), *B. tuberculosis,* human type, and *B. smegmatis* (Moeller). Cultures were grown upon glycerin agar slants and saline suspensions of these, were diluted so as to contain approximately one billion bacilli per cc. They were then killed by autoclaving at 1 atmosphere pressure for 15 minutes. These suspensions were employed for the primary sensitizing and testing injections. Upon 12 guinea pigs and 17 rabbits approximately 100 tests have been made. The guinea pigs used primarily did not present much testing area and the color of the skin detracted from the readings. White rabbits were found preferable, inasmuch as reactions showing redness and tumefaction could be more readily discerned. The area of the chest and abdomen of this animal, also, afforded better space for multiple testing. A series of rabbits was given a subcutaneous sensitizing dose of 2 cc of each microörganismal species respectively, of the above described suspensions. After a period of 21 days the abdomen and thorax were shaved and all animals were tested endermally with .04 cc of the 4 separate allergens. At the same time control animals were similarly tested. Within 48 hours there had developed in the sensitized animals, markedly elevated and reddened nodules in certain of the tested areas. The controls at this time showed no appreciable response. The composite results of such tests are shown in Table I.

In the reactions noted, it was apparent that each allergen showed some degree of relative specificity for the animal homologously sensitized. Later, the differentiation became somewhat obscured and the control developed small nodules. The allergens were then diluted 1-100 and 1-1000 and the same animals tested with these dilutions. Again there were definite evidences of specific reactions which endured for some time.

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[12] Cooke, J. V., *J. Inf. Dis.*, 1919, **25**, 452.

TABLE I.
Endermal Allergic Reactions.

	Allergens			
Sensitized Animals	*B. lepræ* Chrome (Duval)	*B. lepræ* (Kedrowsky)	*B. smegmatis* (Moeller)	*B. tuberculosis* (Human)
Rabbit A *B. lepræ* Chrome (Duval)	++++	++	±	+
Rabbit B *B. lepræ* (Kedrowsky)	—	+++	++	—
Rabbit C *B. smegmatis* (Moeller)	±	+	+++	—
Rabbit D *B. tuberculosis* (Human)	—	±	—	++
Control rabbit	—	—	—	—

++++ = Nodular reaction, approximately 15 mm diameter.
+++ = ” ” ” 10 ” ”
++ = ” ” ” 7 ” ”
+ = ” ” ” 3 ” ”
All showed corresponding degrees of redness, oedema and necrosis.

These experiments were repeated with more white rabbits which were sensitized and after 24 days together with controls, were tested endermally with the higher dilutions of bacterial allergens. Similar differential reactions were obtained in this series as in the former group, with practically negative results in the control animals. A delayed smaller nodule formation was attributed to foreign protein retained in the skin.

These results indicate that this method may prove of service in identification of some one or more strains of *B. leprae* as such. With improvement in the preparation of the testing allergen and the inclusion of an allergen prepared from bacilli of a leprous nodule, a causal relationship may be emphasized.

10115

A Tonometer for Measurement of Tissue Turgor in the Human Finger Tip.*

George E. Burch and William A. Sodeman. (Introduced by R. H. Turner.)

From the Department of Medicine, School of Medicine, Tulane University of Louisiana, and the Charity Hospital of Louisiana, New Orleans.

Because of the frequency of involvement of the finger tip in vascular disease, a method to measure quantitatively physical changes in the finger tip is needed. We have devised for this purpose a mechanical palpator, based upon the principle of the Schioetz tonometer and capable of detecting quantitatively small changes in tissue turgidity. Heretofore only rough estimation of these changes could be made by simple palpation. Schade and others[1, 2, 3] have employed

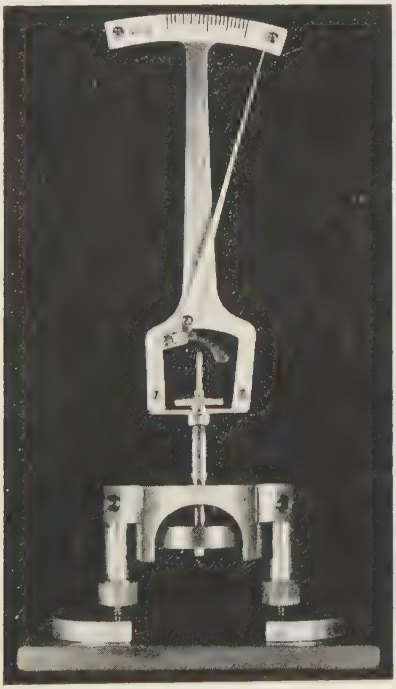

Fig. 1.
Figure showing apparatus mounted for use.

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[1] Schade, H., *Z. f. exp. Path. u. Therap.*, 1912, **11**, 369.

[2] Inouye, K., *Acta Scholæ Med.*, 1931-32, **14**, 229.

[3] Rondelli, N., *Minerva Med.*, 1934, **1**, 810.

related principles in their elastometer studies in edema in other parts of the body.

The apparatus (Fig. 1) is small, simple, accurate and easily applied. It consists of the head of a Schioetz tonometer adapted to a specially constructed base (Fig. 2) so that when the plunger which protrudes through the lower end of *a* (Fig. 2) is forced upward and flush with the lower circular surface of *a* by the application of a flat rigid surface, the pointer will register zero on the scale. The distance between parts *f* and *g* is made equal to the distance to which the plunger protrudes through the base of *a*. This distance is maintained by a spring mechanism *i* which also permits the entire apparatus to be brought down until parts *g* and *f* are approximated.

In making a determination, we place the patient in a sitting position with the forearm resting at heart level comfortably upon a table. The patient's arm is then adjusted so that the wrist is dorsiflexed and the finger to be studied rests with the dorsal surface of the two terminal phalanges upon a glass slab (see Fig. 1). The apparatus is then placed upon the glass slab over the finger tip so that the plunger rests directly above the most prominent point of the finger pad. Part *a* is then screwed up or down in part *b* until the distal end of the plunger just touches the surface of the finger pad.

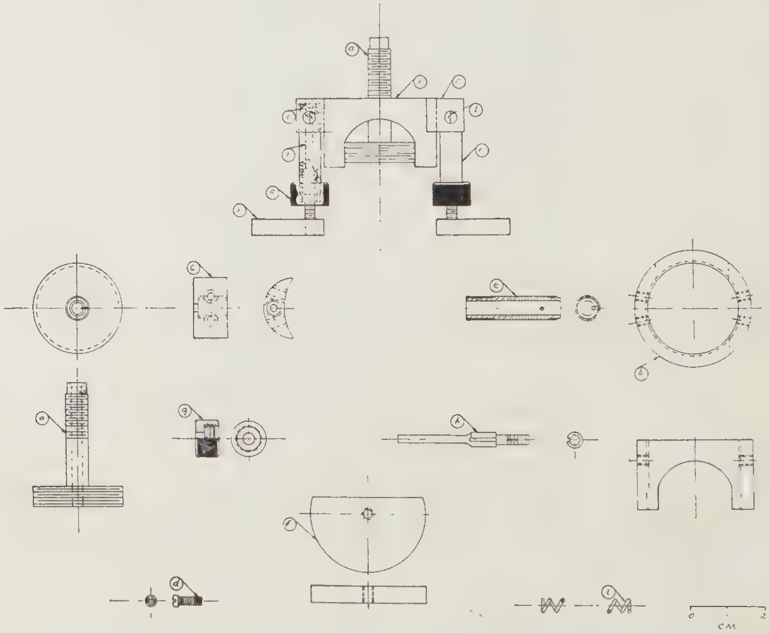

FIG. 2.

Details of construction of the base of the tonometer (all parts brass except steel spring, *i*).

Fine adjustment is permitted by the fine threading of the base of part *a* (80 to the inch). Pressure is then applied on part *b* until part *g* approximates part *f*. This permits the plunger to rest freely upon the finger tip.

It is obvious that if the tissues of the finger pad were perfectly rigid the end of the plunger would be forced upward until the pointer would register zero upon the scale. Since the tissues are not perfectly rigid and there are variations in tissue turgor, the plunger will sink into the tissues, compressing them until the resistance of the tissues equals the weight of the plunger. Therefore, the pointer will come to rest at some point upon the scale above zero, depending upon the tissue turgor, and these variations in scale readings may be used as quantitative expressions of tissue turgidity.

No movement of the hand or finger tip is permitted during a determination. In all determinations at least 3 readings were made which agreed within ±0.5 units.

We have made determinations in the finger tips of 25 normal individuals and 20 patients with vascular disease. The 5.5 g tonometer weight was used in all determinations. In normal subjects tonometer scale readings varied from 15 to 20+ units. The values did not correlate with age. In 9 of the 25 subjects, the tissue turgor of the finger tip was too low to register upon the scale. Such readings indicate that in these individuals the counter force offered by the tissues when compressed by the 5.5 g force through that distance is less than 5.5 g. Such readings are indicated as 20+. In 12 subjects the epidermis was removed with very fine emery paper until the skin rugae were smoothed out. There was no significant difference in the determinations before and after this procedure, thus indicating that the epidermis plays a minor rôle in the values obtained in normal subjects. Repeated determinations in the same individual over periods as long as 6 weeks have shown no significant variations. The tonometer readings were found to vary with position of the finger with reference to the heart. Above heart level, they were higher, and below heart level, lower.

In 20 patients with abnormal vascular states, some striking changes have been noted. In scleroderma, for example, the values at times approached absolute rigidity. In 3 patients with marked involvement of the fingers, values varied from 1.0 to 9.0 (mean 4.2) tonometer scale units. In one patient with early changes the value was 16. In Raynaud's disease the deviations from normal were not as marked. In 3 patients determinations varied from 10 to 13 scale units. In 2 patients with unilateral edema, the affected side showed readings which were definitely lower (10.5, 3.5) than the unaffected side (15, 14). Similar results were found in a patient with bone felon (affected side 10.5, unaffected side 20+). The changes found in edema are well exemplified in the patient with cold allergy. The patient's hand was immersed in cold water (6°C) and withdrawn from time to time for readings. The course of the turgidity of the part is shown in Fig. 3. The volume of the finger tip increased from 3081 to 3489 mm^3, a change of 13.2%. In essential hypertension, arteriosclerosis, and clubbed fingers, less definite changes were noted.

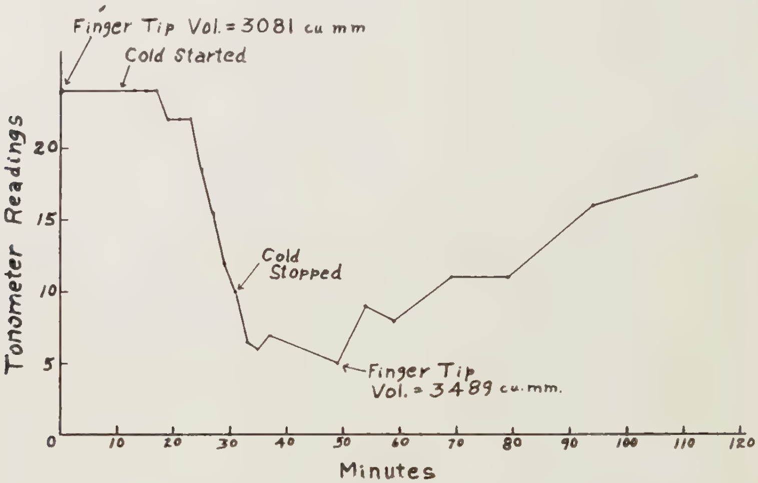

FIG. 3.

Course of tissue turgor of left index finger pad in a patient with cold allergy. The finger tip volumes were determined by a method previously described.[4]

Changes in tissue turgor of the finger pad can be quantitatively estimated by the tonometer, acting essentially as a sensitive mechanical palpator. The instrument measures the distance the plunger must traverse in order to compress the tissues sufficiently to produce a counter force equal to 5.5 g. This distance is determined by the firmness of the tissues and their ability to be displaced. Changes in these properties produced by physiologic and pathologic states may be detected quantitatively by this method and long before the ordinary methods of physical examination disclose such changes. For example, variations in these properties produced by change in position in the part above and below the heart level are easily recorded. Were the finger tip a sphere and encased in a relatively non-distensible envelope, as the eye, one could convert the tonometer scale readings into millimeters of mercury with the use of the Schioetz tonometer conversion scale. However, because of the physical characteristics of the finger tip, this cannot be done.

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[4] Sodeman, W. A., *J. Clin. Invest.*, 1937, **16**, 787.

Remarkable changes in tonometer values were found in patients with diseases affecting chiefly the corium and subcutaneous tissues. This is well illustrated in scleroderma, the trophic changes of Raynaud's disease and edema. The well known increase in connective tissue and in its compactness in the former 2 diseases and the increase in interstitial fluid in the latter are sufficient to explain the increased firmness and turgidity of the finger tip found in these conditions. Since the method can be used to detect these changes, it may be used to follow objectively the progress of these conditions with or without treatment. This is particularly true early in the disease or later when changes are slight and ordinary methods of physical examination inadequate.

No significant changes could be attributed primarily to the epidermis except perhaps in 2 patients with arteriosclerosis and calloused hands. Their values of 9 and 10.5 contrast definitely with the values of 19, 20, 20+, and 20+ in remaining patients with arteriosclerosis who showed no epidermal thickening.

Conclusions. The application of a simple and accurate tonometer to the study of the tissues of the finger tip disclosed findings which may be used to follow the course of certain physiologic and pathologic states.

10116

Absorption of Cholecystokinin and Secretin from the Colon and Rectum.

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This work was undertaken to ascertain whether cholecystokinin and secretin may be absorbed from the colon and rectum in significant functional quantities. If so, these hormones might be so administered and serve as functional tests.

A preparation of cholecystokinin (CCK) and secretin was used which contained one dog unit of cholecystokinin in 0.3 mg and of secretin in 0.1 mg. This was administered per rectum to dogs and human subjects.

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* Ralph Colp Fellow in Physiology, the Mount Sinai Hospital.

The dogs, 5, were prepared for a record of intra-gallbladder pressure and the principal pancreatic duct was cannulated, the accessory duct being tied. Before the dogs were anesthetized, the colon was lavaged to remove all fecal material. The colon of the human subjects, 5, was likewise lavaged.

One hundred mg of the hormone—(1000 units of secretin and 333 units of CCK)—containing preparations were dissolved in normal saline buffered at pH 6.0. The gallbladder did not respond. The flow of pancreatic juice was definitely augmented in 4 of the 5 dogs. In dog 1, the basal flow was doubled; in dog 2, no effect was obtained; in dog 3, a 3-fold increase in flow occurred; in dog 4, the flow increased from a basal of 1 drop in 10 minutes to 1.5 drops per minute; in dog 5, the flow increased from a basal of 1 drop in 30 minutes to 1 drop in 3 minutes. A solution (30 cc) of 25% alcohol, containing 100 mg of the hormone, caused a 6-fold increase in rate of flow; but on performing suitable controls, it was found that the alcohol stimulated the flow of pancreatic juice, and it could not be shown definitely that the alcohol favored the absorption of secretin.

The effect of the hormone preparation on the 5 human subjects was studied by the method of cholecystography; the effect on the pancreas was not studied. The gallbladder was visualized and the colon evacuated by enema before the first X-ray film was made. The gallbladders concentrated well. Then 1.5 g of the hormone preparation (15,000 dog units of secretin and 4,500 units of CCK) dissolved in 200 cc of a saline buffer (pH 6.0) were instilled into the rectum. Films were then made at intervals for 6 hours. In 2 of the 5 subjects the gallbladder decreased in size slightly, but not significantly. In one of the subjects whose gallbladder did not decrease in size after the rectal instillation of the preparation, the test was repeated with negative results.

Summary. Secretin may be absorbed to a slight extent from the dog's colon when administered in relatively enormous doses. Cholecystokinin was not absorbed. Cholecystokinin (4,500 dog units) when placed in the rectum of man is not absorbed to any significant extent.

10117 P

Peroxidase-like Activity of Alloxan and Aldehyde.

Henry R. Jacobs.

From the Department of Medicine, University of Chicago.

Alloxan has recently been found to cause hypoglycemia in normal rabbits.[1] The manner in which this hypoglycemia is brought about should now be explained. This paper presents a reaction of alloxan which might take place in tissues and thus could be concerned with the hypoglycemic effect.

If alloxan in aqueous solution is mixed with a simple aldehyde and hydrogen peroxide, the resulting solution will oxidize benzidine, *p*-phenylenediamine, pyrogallol, iodides, etc. A mixture of alloxan and aldehyde therefore has *peroxidase-like* activity.

The reaction is easily demonstrated. To a few cc of a dilute aqueous solution of an aldehyde add a little alloxan and a few drops of 5% hydrogen peroxide. Then add the substance to be oxidized. For example, 5 drops of a saturated glacial acetic acid solution of benzidine turns the mixture deep blue at once. The reaction will not occur when one of the 3 reagents: alloxan, peroxide, aldehyde, is absent. (Except that lower aldehydes after standing exposed to air and used in strong solution themselves oxidize benzidine.[2]) The reaction will not occur if alloxan is briefly treated with excess alkali, then acidified again. This procedure, which destroys alloxan, eliminates the possibility of metallic catalysis, by traces of copper or iron for example.

All of the aliphatic aldehydes tested and most of the aromatic aldehydes react positively. All substances found to react positively are aldehydes.

Positive. Formaldehyde, hexamethylene tetramine, trioxymethylene (slowly), acetaldehyde, paraldehyde, propionaldehyde, n-butyl aldehyde, crotonaldehyde, aldol, glyceric aldehyde, glyoxal, methyl glyoxal, benzaldehyde, piperonal.

Negative. Ethyl alcohol, acetone, ether, chloroform, glycine, alanine, glycine anhydride, sodium butyrate, glycerol, sodium glycerophosphate, urea, glucose, galactose, sucrose, glycogen, arabinose, xylose, taurine, creatine, creatinine, phlorhizin, chloranil, xanthine, chloral hydrate, *p*-dimethylaminobenzaldehyde, salicylaldehyde, and many others.

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[1] Jacobs, H. R., Proc. Soc. Exp. Biol. and Med., 1937, **37**, 407.

[2] Woker, G., *Z. Allg. Physiol.*, 1914, **16**, 341; Gallagher, P. H., *Bioch. J.*, 1924, **18**, 29.

As substitutes for alloxan, barbituric acid, isobarbituric acid, barbital, nembutal, alloxantin, murexide, parabanic acid, uric acid, diketopiperazine, chloranil, uramil and cyanuric acid were worthless. No substitutes for simple peroxides were found.

The reaction is very sensitive for the simpler aldehydes. Formaldehyde can be detected in 1-50,000 dilution. The reaction is prevented by very dilute solutions of hydrazines, hydroxylamine, cyanide, bisulfite and cysteine. It is not affected by alcohols and amino-acids. In many respects, therefore, the resemblance to plant and animal peroxidase preparations is great.

Whether the peroxidase-like activity of alloxan and aldehyde is the basis for the observed hypoglycemic effect of alloxan is not known. All of the required reagents are in the tissues. Hydrogen peroxide is formed by the oxidases and by the direct oxidation of sulphydryl compounds and other substances. Aldehydes are present normally.[3] The substrates to be oxidized remain undistinguished in a large group. One surmises that per-acids are formed from aldehydes *in situ* and that they are active in an oxidative process which removes sugar from the blood. Crude peroxidase from milk[4] causes hyperglycemia only, as might be anticipated from its high protein content.

The reaction here described may help explain the acceleration of oxygen uptake by minced liver when treated with alloxan as noted by Bernheim,[5] the peculiar reactivity of aldehydes in biological reactions, and a possible function of hydrogen peroxide in the tissues.

Summary. Alloxan, aldehydes, and hydrogen peroxide together appear to form an oxidizing substance more reactive than any one or two of these reagents alone. This peculiarity results in a peroxidase-like oxidizing system and a specific test for aldehydes.

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[3] Supniewski, J. V., *J. Biol. Chem.*, 1926, **70**, 13.

[4] Elliott, K. A. C., *Bioch. J.*, 1932, **26**, 10.

[5] Bernheim, F., *J. Biol. Chem.*, 1938, **123**, 741.

10118 P

Homoplastic Transplantation of Suprarenal Glands of Rat into the Anterior Chamber of Eye.

C. Donnell Turner. (Introduced by Hans O. Haterius.)

From the Department of Zoology, Northwestern University.

It has been established that functional suprarenal grafts consist only of cortical tissue, the medullary part of the gland seldom persisting. It is known that the interior of the transplants degenerates after the blood supply is interrupted and that the new cortex is restored by proliferation from a peripheral layer of glomerulosa or by the differentiation of unspecialized cells in the capsule.[1] Incorporation of the cortex does not occur subsequent to the removal of the capsule.[1, 2] Previous workers have reported that neither autoplastic nor homoplastic suprarenal transplants in intramuscular and intraperitoneal positions persist in hosts possessing both or one of the intact suprarenal glands.[1, 3]

The present experiments were begun in order to analyze the factors which regulate the incorporation and persistence of suprarenal transplants. It was thought that the anterior chamber of the eye should be a more favorable transplantation site than those which had been previously employed. Whole glands from young donors, ranging in age from birth to 32 days, were transplanted unilaterally and bilaterally to the eyes of adult hosts. A total of 387 glands were introduced into the eyes of 245 hosts. The grafts were removed at intervals ranging from 12 hours to 5 months after insertion and studied histologically and cytologically.

Among normal hosts, a total of 51% of the bilaterally transplanted glands incorporated and contained cortical tissue at the time of removal; 64% of the unilaterally transplanted glands persisted under the same conditions (Table I a). While cortical proliferation was not so extensive in grafts from normal hosts as in those from doubly suprarenalectomized animals, these experiments are sufficient to demonstrate conclusively that cortical restoration does occur in intra-ocular grafts in rats possessing both intact suprarenal glands. A significantly higher percentage of transplants persisted in suprarenalectomized hosts than in normal hosts (Table I a, b). By reference to Table I it may be seen that the unilateral grafts

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[1] Ingle, D. J., and Higgins, G. M., *Endocrinology*, 1938, **22**, 458.

[2] Wyman, L. C., and Walker, B. S., *Am. J. Physiol.*, 1929, **89**, 215.

[3] Wyman, L. C., and tum Suden, C., *Am. J. Physiol.*, 1932, **101**, 662.

TABLE I.
Summary of Incorporated Suprarenal Transplants.*

Bilateral Transplantation				Unilateral Transplantation			
Type of host	No. of transplantations	No. of grafts incorporated	% incorporated	Type of host	No. of transplantations	No. of grafts incorporated	% incorporated
a. Entire glands to normal hosts.							
♀	56	30	53.6	♀	27	18	66.7
♂	40	19	47.5	♂	12	7	58.3
total	96	49	51.0	total	39	25	64.1
b. Entire glands to doubly suprarenalectomized hosts.							
♀	24	22	91.7	♀	12	12	100.0
♂	88	78	88.6	♂	36	33	91.7
total	112	100	89.3	total	48	45	93.7
c. Entire glands to normal hosts plus pituitary implants.							
♀	30	22	73.3	♂	2	2	100.0
d. Capsules of suprarenal glands to suprarenalectomized hosts.							
♀	16	11	68.7	♂	4	4	100.0
e. Medullæ to normal hosts.							
♀	22	6	27.3	♀	5	0	0

* Fibrotic or calcified nodules were not counted as incorporated grafts. The transplants removed during the first 10 days after insertion into the eye are not included in the table.

persisted better than the bilateral ones. Histological studies indicated that there was more extensive cortical proliferation in the grafts from suprarenalectomized hosts than in those from normal hosts, and also that there was more extensive cortical restoration when the glands were transplanted unilaterally. The percentage of incorporated grafts recovered from normal hosts receiving pituitary homo-implants was greater than that recovered from comparable hosts which were not implanted (Table I a, c). The size of the grafts from implanted hosts, as well as the degree of cortical regeneration, did not equal that of grafts persisting in doubly suprarenalectomized hosts but was much in excess of that characteristic for normal untreated hosts. No pronounced sex difference in the capacity to regenerate cortical tissue, as reported by Wyman and tum Suden,[3] was observed in the intra-ocular grafts.

After preparing the glands according to the technic of Ingle and Higgins,[1] enucleated capsules (*i. e.*, capsular investment separated from cortical and medullary tissue) were transplanted to the anterior eye chamber and recovered after persistence for approximately 2 months. The results are summarized in Table I d. Medullary tissue was expressed from the glands of 20-day-old donors and, while the glands were in Ringer's solution, the adherent cortex was further teased away under a dissecting microscope. The medullæ were transplanted to normal adult hosts and 27% of them were recovered and found to contain histologically normal medullary cells after persisting for 20 days (Table I e).

Summary. Homoplastic suprarenal cortices may incorporate and persist for as long as 5 months in the eyes of normal hosts. The extent of cortical regeneration in suprarenal grafts as well as the frequency of persistence is augmented by suprarenalectomy and by homopituitary implantation. The evidence seems to indicate that differentiation in cortical transplants is conditioned by the cortico-adrenotropic principle of the anterior hypophysis. The medullary tissue is relatively specialized but it may persist in homoplastic grafts if the period of nutritional interruption is minimized.

10119

Effect of Beta-methylcholine-urethane on Normal and on Reflexly Inhibited Intestine.*

W. B. Youmans and R. C. Waisman. (Introduced by W. J. Meek.)

From the Department of Physiology, University of Wisconsin Medical School, Madison.

Although acetylcholine produces marked stimulation of the isolated intestine,[1] it is not suitable for increasing the motility of the intestine *in situ* because of its rapid hydrolysis by the cholinesterase of the blood,[2] its severe blood-pressure lowering effect, and its indiscriminate stimulation of autonomic ganglia (nicotinic action). Protecting acetylcholine from hydrolysis by the use of physostigmine potentiates and prolongs the intestinal effects of acetylcholine[3] but the other two objections are still applicable. Acetyl-beta-methylcholine lacks the nicotinic action[4] and is somewhat more stable in blood, but it also has severe blood pressure effects due to cardiac slowing especially when given parenterally.[5] A sharp fall in blood pressure causes reflex production of adrenine and sympathin, both of which are inhibitors of the intestine. These compensatory responses occur

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* Made possible in part by a grant from the Wisconsin Alumni Research Foundation.

[1] Dale, H. H., *J. Pharm. and Exp. Therap.*, 1914, **6**, 147.

[2] Matthes, *J. Physiol.*, 1930, **70**, 338.

[3] Zimmerman, L. M., Frank, R., and Necheles, H., *Arch. Surgery*, 1936, **33**, 187.

[4] Simonart et Simonart, *Arch. internat. de Pharm. et de Therap.*, 1935, **51**, 76.

[5] Starr, I., *Trans. Assn. Am. Physicians*, 1936, **51**, 326.

following injection of blood-pressure lowering choline derivatives.[6] The carbamic esters of choline and of beta-methylcholine which are called carbaminoylcholine and beta-methylcholine-urethane (carbaminoyl-beta-methylcholine) are comparatively stable in blood and the latter has the additional advantage of lacking the nicotinic action.[4] Carbaminoyl choline was given by Bosse[7] to patients having atonic bladders and intestines. In 12 patients with mechanical ileus treated with carbaminoyl choline he found increased peristalsis, indicated by borborygmi followed by the passage of flatus and stools. However, the compound has some undesirable side actions and possesses the nicotinic action. Simonart has concluded that, other properties being equal, a choline compound free from the nicotinic action should be preferable for clinical application, and the major effect of introducing the beta-methyl group is to eliminate the nicotinic action. Starr[5] used beta-methylcholine-urethane on several patients with postoperative abdominal distention and reported favorable results. He stated that when given in ascending doses the first painful effects were cramps and urethral spasm without significant effect on blood pressure. Molitor[8] found the compound to produce defecation in unanesthetized dogs. Farber[9] found that a small intravenous dose causes a fall in blood pressure due to vasodilatation, but the dose must be quite large before heart slowing is produced. These facts suggest that beta-methylcholine-urethane (b-m-c-u) lacks the characteristics which make acetylcholine and many other choline derivatives unsuitable for use in increasing the tonus and activity of the intestine. We have studied the effects of b-m-c-u on the motility of the normal and of the reflexly inhibited intestine of unanesthetized dogs.

Intestinal activity was recorded from jejunal segments in the form of Thiry fistulæ. A balloon inserted into the fistula was connected by means of rubber tubing to one arm of a mercury manometer, the other arm of which floated a writing point. Two such systems were used to record simultaneously the activity of innervated and of denervated Thiry fistulæ in the same animal. Four dogs were used for recording effects on intestinal motility. Each of 3 of these dogs had 2 Thiry fistulæ, one of which was innervated and the other denervated by cutting the mesentery and stripping the nerve fibres from the blood vessels. The fourth dog had a single

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[6] Hermann, H., Jourdan, F., Morin, G., and Vial, J., *Arch. internat. de Pharm. et de Therap.*, 1937, **57**, 403.

[7] Bosse, U., *Klin. Wochensch.*, 1937, **16**, 1365.

[8] Molitor, H., *J. Pharm. and Exp. Therap.*, 1936, **58**, 337.

[9] Farber, S., *Arch. internat. de Pharm. et de Therap.*, 1936, **53**, 377.

Thiry fistula which was denervated by removal of the coeliac ganglia and vagotomy above the diaphragm. Rectal stimulation was done by inserting a catheter into the anal canal past the sphincter and moving the catheter back and forth to cause sphincter activity. Direct blood pressure records were taken from the femoral arteries of doubly-vagotomized dogs under ether-morphine anesthesia. Beta-methyl-choline-urethane $(CH_3)_3NClCH_2CH\ CH_3\ OCONH_2$† was given subcutaneously or intravenously.

Effect of b-m-c-u on intestinal motility: In the 4 animals after subcutaneous injections of 0.1 mg/kilo of b-m-c-u the tonus and the amplitude of rhythmic contractions were increased in both the innervated and the denervated intestinal segments. No definite effect was observable when the subcutaneous injection was 0.07 mg/kilo or less. This is about 50% larger than the dose which Starr[5] used. Following subcutaneous injections the initial effects occurred within 3 to 6 minutes and became maximal within 15 minutes. Effects were observable as long as 45 minutes following the injection.

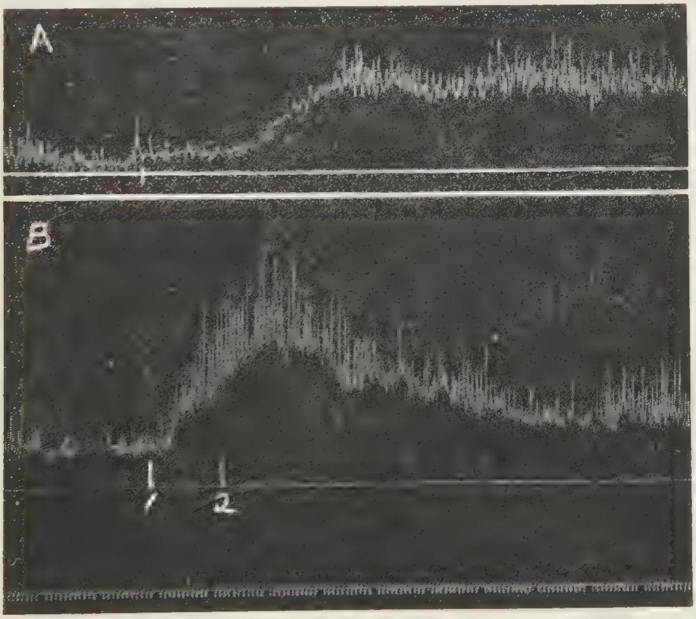

FIG. 1.

Effect of beta-methylcholine-urethane on intestinal tonus and motility. Both A and B are balloon mercury manometer records of motility of the same Thiry fistula on different days. In A, 0.15 mg/kilo of b-m-c-u was given subcutaneously. In B, 0.05 mg/kilo was given intravenously between 1 and 2. Time in 5 seconds and 1 minute intervals.

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† Supplied by Merck & Co., Rahway, N. J.

An intravenous dose of 0.05 mg/kilo of body weight injected within an average period of 75 seconds produced an increase in tonus of from 9 to 24 mm of Hg and an increase in the amplitude of rhythmic contractions in each of 6 cases. The tonus increase began within one minute after beginning the injection and lasted from 9 to 15 minutes. Fig. 1 shows a typical response to a subcutaneous (A) and an intravenous (B) injection in the same animal.

The fact that the innervated and the denervated intestine showed the same type of response to the drug suggests that its action is peripheral.

Within a few minutes after subcutaneous injection of 1.0 mg of

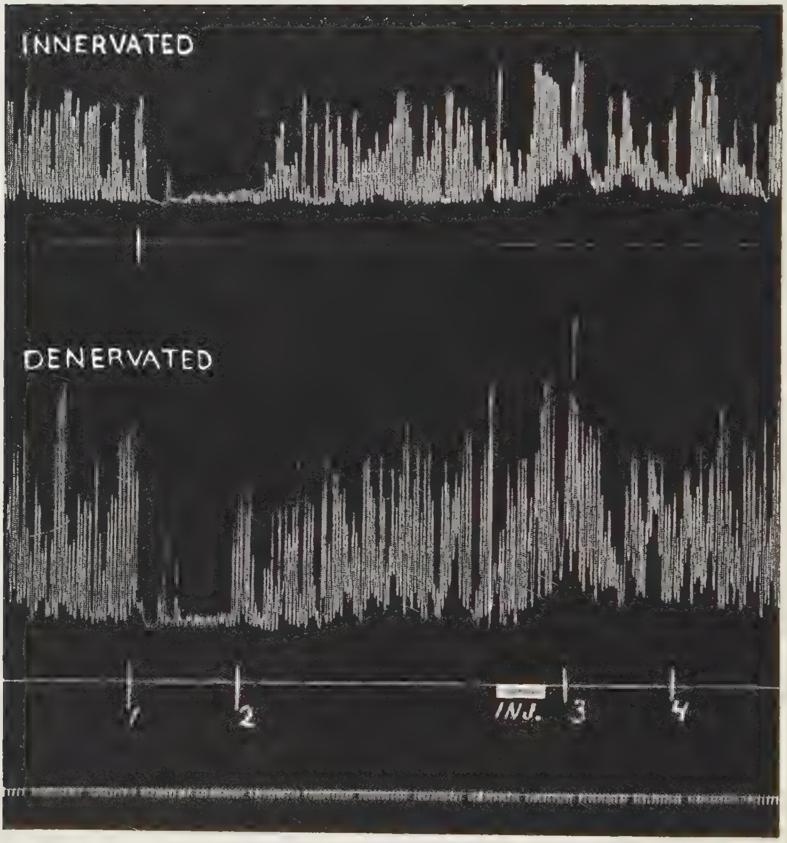

FIG. 2.

Prevention by beta-methyl-choline-urethane of reflex inhibition of intestinal motility. Balloon-mercury-manometer records of activity of innervated and denervated Thiry fistulæ. Rectal stimulation between 1 and 2, also between 3 and 4. Injection at INJ (intravenously) of .04 mg/kilo of b-m-c-u. Time in 5 seconds and 1 minute intervals.

atropine neither the subcutaneous nor the intravenous administration of the above doses of b-m-c-u produced any effect on intestinal motility.

Rectal stimulation causes reflex inhibition of the tonus and rhythmic contractions of the intestine. The reflex inhibition thus produced utilizes sympathetic nerves, the activation of which results in the entrance of sympathin into circulation so that a denervated intestinal segment is inhibited during rectal stimulation.[10] It has been indicated that sympathetic nerves are utilized in reflex paralytic ileus.[11] B-m-c-u when given intravenously, completely opposed the inhibitory effects of rectal stimulation on both the innervated and the denervated intestine. Fig. 2 shows a typical record. Intestine-inhibiting effects of rectal stimulation were only partly prevented by the relatively small subcutaneous doses given. These facts support the opinion that b-m-c-u might be useful in opposing reflex paralysis of the intestine.

Results of subcutaneous injections of b-m-c-u into 8 stock dogs (dosage 0.10 to 0.30 mg/kilo) were similar to those reported by Molitor.[5]

Blood-pressure effects of intestine-stimulating doses of b-m-c-u: In experiments on 8 vagotomized dogs under ether-morphine anesthesia the following facts were observed. 1. Subcutaneous doses ranging from the same to 40% greater than the doses used in the studies on intestinal activity had no effect on mean arterial blood pressure. 2. Intravenous doses ranging from 0.007 to 0.15 mg/kilo/min produced marked but transitory decreases in blood pressure, but without decreasing the heart rate. 3. When an intravenous injection was maintained at a constant rate for a period of 10 minutes, recovery from the initial drop in pressure occurred. For example, in one case using a dose of 0.007 mg/kilo/min the blood pressure dropped from 128 to 102 mm/Hg within 15 seconds, then after one minute the pressure rose to 118 mm/Hg and was maintained at that level until the end of the injection. If the blood pressure changes appearing in these acute experiments are indicative of what occurs in the unanesthetized animal, a subcutaneous dose of b-m-c-u sufficient to cause increased tonus of the intestine is without blood pressure effects.

Summary. Beta-methylcholine-urethane, a drug which has been reported to have beneficial effects in cases of postoperative abdominal distention, causes a marked increase in the tonus and the amplitude

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[10] Youmans, W. B., and Meek, W. J., *Am. J. Physiol.*, 1937, **120**, 750.

[11] Markowitz, J., and Campbell, W. R., *Am. J. Physiol.*, 1927, **81**, 101.

of rhythmic contractions of innervated or of denervated jejunal segments of unmedicated dogs. The drug opposes inhibition of the intestine by reflexly activated sympathetic nerves or inhibition by the sympathin released as a result of the activity of these nerves. The effects of the drug on intestinal motility are prevented by atropinization.

10120

Effect of Anol and Dihydrotheelin on Mammogenic Activity of Pituitary Gland of Rabbits.*

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From the Department of Dairy Husbandry, Missouri Agricultural Experiment Station.

It has been shown that estrogen stimulates the production of a mammogenic hormone entity by the anterior pituitary which is directly responsible for the growth of the mammary gland.[1, 2] More recently, Gomez and Turner[3] reported that anol stimulated the growth of the mammary glands of ovariectomized rabbits, rats and male mice and suggested that like estrogen, the action of anol upon the mammary gland is probably indirect by way of the pituitary gland. It is interesting, therefore, to determine the effect of various estrogens and chemical substances having the dual activity of estrogenesis and carcinogenesis upon the mammogenic activity of the anterior pituitary gland. In the present communication, the effect of anol and dihydrotheelin is reported.

A group of 8 young adult virgin female rabbits were each given by subcutaneous injection once daily 0.1 mg of anol‡ for 25 days:

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* Aided in part by a grant from the International Cancer Research Foundation.

† Contribution from the Department of Dairy Husbandry, Missouri Agricultural Experiment Station, Journal Series No. 577.

[1] Gomez, E. T., Turner, C. W., and Reece, R. P., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 286.

[2] Gomez, E. T., and Turner, C. W., *Mo. Agr. Exp. Sta. Res. Bul.*, 1937, 259; PROC. SOC. EXP. BIOL. AND MED., 1938, **37**, 607.

[3] Gomez, E. T., and Turner, C. W., 1938, in press.

‡ The anol used in this study was prepared by the British Drug Houses, Ltd., London. This anol contains 10 rat units of estrogenic activity per milligram of the crystalline material.

a second group of 10 rabbits with 20 rat units of dihydrotheelin daily for 25 days and a third group of 6 rabbits with 0.1 mg of anol and 20 rat units of dihydrotheelin simultaneously once daily for 25 days. All injected animals were sacrificed 24 hours after the last injection. In addition, a group of 24 young adult virgin females and another of 10 young adult male rabbits were sacrificed as control donors. The pituitaries of animals from each group were combined, weighed and assayed as a unit for mammogenic activity by the male mice technic described by Lewis, *et al.*[4] According to this method of assay, a mouse unit of mammogen is defined as the minimum amount of pituitary tissue or extract which when injected once daily over a period of 6 days will produce definite mammary growth stimulation of one or more glands of 50 ± 10% of 10 male mice each weighing 10 to 25 g.

TABLE I.
Effect of Anol and Dihydrotheelin on Mammogenic Activity of Anterior Pituitary Gland of Rabbits.

			No. of test mice	
Condition of donor rabbits	Total dosage per test mouse mg of pituitary	No. of test mice used	Showing mammary stimulation (+)	Showing no mammary stimulation (—)
Normal young adult females	20	4	0	4
	40	4	0	4
	80	5	0	5
Normal young adult males	60	4	0	4
Young adult virgin females injected with 0.1 mg of anol daily for 25 days	3	2	0	2
	6	2	0	2
	9	2	0	2
	12	3	0	3
	15	5	4	1
	13	10	6	4
Young adult virgin females treated with 20 rat units of dihydrotheelin daily for 25 days	6	2	0	2
	9	2	0	2
	12	2	0	2
	15	2	0	2
	18	2	0	2
	20	10	5	5
Young adult virgin females treated with .1 mg of anol and 20 r.u. of dihydrotheelin simultaneously daily for 25 days	3	2	0	2
	6	2	0	2 (?)
	9	3	2	1
	7.5	8	5	3

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[4] Lewis, A. A., Turner, C. W., and Gomez, E. T., *Endocrinology*, 1938, in press.

A summary of results of the assay of each group of pituitaries is given in Table I. It may be seen from this table that 12 to 13 mg of fresh pituitary tissue from rabbits which had received 25 daily injections of anol was required to establish a mouse unit of mammogen; whereas 20 mg was required with pituitaries from animals which had received 20 rat units of dihydrotheelin daily for 25 days. Seven to 7.5 mg of fresh pituitary tissue from rabbits which had received 0.1 mg of anol and 20 rat units of dihydrotheelin simultaneously daily for 25 days was required to establish a mouse unit. Dosages as high as 60 mg and 80 mg of fresh pituitary tissue from adult males and young virgin female rabbits respectively, were ineffective in stimulating the growth of the mammary glands of male mice. These results were interpreted as indicating that the pituitary of normal adult males and young adult virgin female rabbits contain little or no mammogen.

Both the anol and dihydrotheelin definitely stimulate the production of mammogen by the pituitary gland of rabbits. Though the relation of the anol and dihydrotheelin to the production of mammogen has not been established in this experiment, the present results seemed to indicate that at the dosage level administered anol was more effective insofar as the stimulation of the production of mammogen by the pituitary gland is concerned. It is interesting to note that anol is capable of inducing growth in both the ducts and lobule-alveolar systems of the mammary gland.[3] The simultaneous injections of anol and dihydrotheelin considerably increases the mammogenic activity of the pituitary gland of rabbits as compared to the pituitaries of animals which had received either anol or dihydrotheelin alone. Further studies to determine the effect of dosages and length of administration of anol and dihydrotheelin as well as other naturally occurring and synthetic estrogens are now in progress.

10121

Phlorhizin Glycosuria and Hypoglycemia Unaffected by Excess Ingestion of Na and K Salts.

Mildred R. Ziegler and Irvine McQuarrie.
From the Department of Pediatrics, University of Minnesota.

The daily ingestion of sodium salts in large amounts (1 to 2 g per kg of body weight per day) was found by Thompson, McQuarrie and Anderson[1, 2] to increase the ability of diabetic children to utilize carbohydrate, as shown by a marked decrease in glycosuria and a gradual lowering of the fasting blood sugar level. Corresponding potassium salts were found to exert a diametrically opposite effect on carbohydrate metabolism, one part of potassium counteracting the effect of approximately 3 chemically equivalent parts of sodium. The beneficial effect of a high intake of sodium chloride was confirmed in adult diabetic subjects by MacLean[3] and by Sanstead.[4] More recently Crabtree and Longwell[5] have partially explained the action of the excessive intake of sodium salt by their demonstration that it causes a marked increase in the deposition of liver glycogen in young rats. On the other hand Silvette and Britton[6] have recently shown that potassium in large amounts tends to interfere with the deposition of liver glycogen. That potassium is involved in the mechanism of normal carbohydrate metabolism is further suggested by the fact that its level in the blood plasma tends to follow that of glucose, being reduced markedly by the injection of insulin.[7, 8] In a study on the influence of phlorhizin on the inorganic metabolism in dogs, Kastler[9] found that the serum potassium was decreased while the inorganic phosphorus was increased following the administration of this glucoside.

The present investigation was undertaken with the hope of shedding additional light upon the apparent interrelationship between carbohydrate metabolism and the economy of sodium and potassium. The direct object of the experiments was to determine the specific effects of NaCl and of KCl on phlorhizin glycosuria and hypoglycemia when given separately in excessive amounts. Four normal dogs kept in individual metabolism cages were given basal maintenance diets, consisting of lard 2.0, butter fat 1.0, sucrose 2.5, whole wheat flour 3.0, artificial "bone ash" 0.4, egg white 2.0, and

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[1] McQuarrie, I., and Thompson, W. H., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 907.

[2] McQuarrie, I., Thompson, W. H., and Anderson, J. A., *J. Nutrition*, 1936, **11**, 77.

[3] MacLean, A. R., *Proc. Staff Meet. Mayo Clin.*, 1935, **10**, 321.

[4] Sanstead, H. R., *Hospital News*, 1936, **21**, 1.

[5] Crabtree, D. G., and Longwell, B. B., PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 705.

[6] Silvette, H., and Britton, S. W., PROC. SOC. EXP. BIOL. AND MED., 1937, **37**, 252.

[7] Briggs, A. P., Koechig, I., Doisy, E. A., and Weber, C. J., *J. Biol. Chem.*, 1923, **58**, 721.

[8] Harrop, G. A., and Benedict, E. M., *J. Biol. Chem.*, 1924, **59**, 683.

[9] Kastler, A. O., *J. Biol. Chem.*, 1928, **76**, 643.

egg yolk 2.0 g per kg of body weight daily. In addition each dog received one-half gram tablet of powdered yeast daily. Drinking water was given *ad libitum.* Two dogs were used for determination of the effects of NaCl and 2 for the KCl experiments. In each instance the time for the experiments was divided into periods of 6 or 7 days each, during which the animal received the diet alone, the diet plus extra salt, the diet plus daily injections of phlorhizin and the latter plus the extra salt. Recovery and post-recovery periods were also included. Phlorhizin was administered subcutaneously in olive oil in single daily doses of 100 mg per kg of body weight. NaCl was given with the diet in enteric coated pills at the rate of 1 g per kg of body weight per day. KCl was given to one dog in this manner and in the same dosage. The second dog to be given KCl received but 0.5 g per kg of body weight daily. The excretion of nitrogen, glucose and minerals was measured for all periods. In addition the non-protein-nitrogen, the sugar and the chief electrolytes of the blood were determined at the beginning of the experiment and at the end of each period, blood samples being obtained after a 14-hour fast.

TABLE I.
Summary of Data Regarding Effects of NaCl and KCl on Phlorhizin Glycosuria and Hypoglycemia.

Experimental Condition	Days	Blood Glucose mg/100 cc	Serum K mg/100 cc	Serum Inor. P mg/100 cc	Glucose Excreted g/kg/day
		Dog 22.			
Diet alone	6	63	18.2	4.8	0
Phlorhizin	7	51	15.3	4.2	4.5
Recovery	7	63	19.7	4.3	0.5
NaCl (1 g per kg per day)	6	56	18.5	4.4	0
NaCl plus phlorhizin	7	55	14.7	4.8	5.5
Phlorhizin	3	—	—	—	5.2
Recovery	10	69	19	4.1	0.2
		Dog 25.			
NaCl (1 g per kg per day)	5	94	17.9	2.8	0
NaCl plus phlorhizin	7	63	12.3	3.3	4.9
Phlorhizin	3	—	—	—	4.3
Recovery	6	80	17.2	3.4	1.5
		Dog 10.			
Diet alone	6	72	17.2	4.6	0
Phlorhizin	7	43	14.3	4.3	5.0
Recovery	7	62	18.4	4.2	2.4
KCl (1 g per kg per day)	5	69	17.5	4.3	0
KCl plus phlorhizin	7	37	18.6	3.7	5.0
Phlorhizin	3	—	—	—	3.3
Recovery	4	69	18.0	3.1	1.1
		Dog 32			
KCl (0.5 g per kg per day)	6	69	19.1	4.5	0
KCl plus phlorhizin	7	55	17.6	4.8	3.9
Phlorhizin	3	—	—	—	5.1
Recovery	8	64	19.6	4.1	3.8

The essential results are summarized in Table I. The effects of phlorhizin alone on the blood constituents were similar to those reported by Kastler, except for the absence of a significant increase in serum inorganic phosphorus. While the serum potassium was regularly reduced by phlorhizin alone, it remained unchanged when phlorhizin administration was accompanied by the ingestion of potassium chloride in large amounts. On the other hand, the reduction of serum potassium as a result of phlorhizin administration was apparently accentuated by the feeding of excessive amounts of sodium chloride. In neither instance, however, did the extra salt ingested significantly influence the degree of phlorhizin hypoglycemia. The glycosuria due to phlorhizin administration was likewise essentially unaffected by either salt. The slight increase observed in some experiments was obviously due to an accentuation of the phlorhizin diuresis by the salt ingested. Slight variations which were noted in the excretion of nitrogen and of minerals could be accounted for in the same way. The calcium, magnesium, sodium, non-protein nitrogen, chloride and CO_2 content of the serum were not significantly altered by the procedures employed.

It is concluded from these experiments that the disturbance in carbohydrate metabolism due to phlorhizin poisoning bears no relationship to that of diabetes mellitus as regards its response to ingestion of excessive amounts of sodium or potassium salts. The characteristic effects of these salts on hepatic glycogenesis in the normal dog appear to be abolished by phlorhizin.

10122

Use of Aluminum Metal in Contact with Blood in Perfusion Systems.

IRENE L. BELAND, GORDON K. MOE AND M. B. VISSCHER.
From the Department of Physiology, University of Minnesota, Minneapolis.

In attempting to use the Bayliss-Fee oxygenator in isolated mammalian heart studies it was found that the life of the preparation was very short, as little as 30 minutes. The coronary flow was found to diminish progressively and the hearts failed rapidly. In investigating possible causes it was found that considerable $Al(OH)_3$ accumulated in the system. The oxygenator is made of cast aluminum. The corrosion of the aluminum metal obviously led to the production of $Al(OH)_3$. Therefore all surfaces were covered with a uniform thin coat of clear lacquer. The life of the isolated heart in the system was thereby greatly prolonged.

To elucidate the mechanism of the effect of aluminum two types of experiments were performed. First, frog hearts were set up on Straub cannulæ and Ringer containing 200-400 mg % of $Al(OH)_3$, as alumina cream, was substituted for the plain Ringer placed in the cannula for control observations. These amounts of $Al(OH)_3$ had no toxic effects upon the frog hearts, rather there was a slight tonic effect and the hearts kept beating longer than did simultaneous controls.

A second group of experiments were run upon rabbit hearts perfused by the Langendorff technic. There was a trace of blood in the perfusion fluid in the first experiments. Addition of 2 cc of 2% $Al(OH)_3$ in Ringer to the perfusion fluid caused almost immediate stoppage of the heart, and of the flow of fluid through the coronaries. In other experiments the heart was perfused with Ringer containing no blood and the perfusate discarded. It was washed with 1500 cc of Ringer, after which the emerging fluid was protein-free by the tungstic acid test. Addition of 15 cc of 2% $Al(OH)_3$ thereafter did not stop the heart, although the amplitude of contraction was somewhat diminished. In still another experiment the coronaries were thoroughly washed with plain Ringer as before. Addition of 40 mg % of $Al(OH)_3$ to the perfusate had a noticeable tonic effect. Perfusion of this solution, oxygenated, was continued for 40 minutes without deterioration. Then 10 cc of the rabbit's blood were added to the $Al(OH)_3$ containing Ringer. At the instant that this fluid reached the heart the beat became feeble and the coronary flow fell off sharply. The heart was dead within 5 minutes.

A single experiment was performed with the ordinary heart-lung preparation in which, first, 8 cc of 2% $Al(OH)_3$ was injected slowly into the blood as it flowed to the right atrium. There was no detectable effect upon the heart. Next 2 cc of the same solution was slowly injected directly into the left atrium. The heart began to fail immediately and stopped beating within 5 minutes. It is apparent that in the first case passage through the lungs removed the toxic agent, and that injection directly into blood entering the coronary arteries was fatal. The lung is an efficient filter and this property probably accounts majorly for the superiority of the heart-lung preparation over isolated heart circuits for cardiac physiology studies.

We conclude that $Al(OH)_3$ is toxic only to hearts perfused through coronary arteries, and that the damage in the latter case is probably due to capillary embolism resulting from the precipitation of plasma protein by $Al(OH)_3$. Obviously, one should avoid the use of aluminum metal in contact with blood in any perfusion systems.

10123 P

Origin of Bone Marrow Plasma Cell Associated with Allergic and Immune States in the Rabbit.*

Fred Kolouch, Jr. (Introduced by E. A. Boyden.)
From the Department of Anatomy, University of Minnesota, Minneapolis.

The observation of a plasmacytosis of the bone marrow of a man with a fatal endocarditis, coupled with Downey's description of a great number of plasma cells in the bone marrow of a rabbit with an incidental, non-specific, subcutaneous abscess suggested an experimental method for increasing the bone marrow plasma cell content. This reaction was also indicated by the bone marrow from rabbits allergic to and immunized against *Streptococcus viridans,* obtained through the courtesy of Dr. B. J. Clawson of the Department of Pathology, University of Minnesota. In these animals it was noted that the allergic and immune animals showed a more extensive medullary plasmacytosis than those that were immune but not allergic. Through the study of the myeloid tissue during the development of a plasmacytophilia, we felt something of the origin and function of the bone marrow plasma cell might be determined.

There are many conflicting theories regarding the origin of the myeloid plasma cell (Downey,[1] Michels,[2] Rohr,[3] Jordan and Morton,[4] and Osgood and Ashworth[5]). The current theories make the lymphocyte, histiocyte, fibroblast, myeloblast, erythroblast, plasmacytic reticulum cell and specific plasmablast the parent of the bone marrow plasma cell.

The procedure we employed was as follows: Obtain a control sample of normal marrow; induce an increase in the plasma cells and follow the bone marrow picture by means of successive biopsies while the increment in plasma cells is in progress. In this manner, we were able to identify the progenitor of these cells during their initial stage of differentiation in the bone marrow.

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* This work was carried out under the direction of Dr. Hal Downey in the Hematological Laboratory of the Department of Anatomy, University of Minnesota.

[1] Downey, Hal, *Folia Hæmat.*, 1911, **11**, 275.

[2] Michels, N. A., *Arch. Path.*, 1931, **11**, 775.

[3] Rohr, K., *Folia Hæmat.*, 1936, **55**, 305.

[4] Jordan, H. E., and Morton, C. B., *Am. J. Anat.*, 1937, **61**, 407.

[5] Osgood, E. E., and Ashworth, C. M., Atlas, 1937, J. W. Stacey, Inc., San Francisco.

We treated a series of 13 rabbits by Clawson's method, *i. e.,* giving a subcutaneous injection of *Streptococcus viridans* broth agar mixture and thus making the animal allergic to this antigen. (Following the injection the agar solidifies on cooling and localizes the organism.) After allergy was induced, intravenous injections of a broth culture of *Streptococcus viridans* were made. The condition of the bone marrow was followed throughout this experimental procedure by means of subtotal costectomies. A control rib was removed before allergy was induced, another in the allergic state and successive ribs after the intravenous injection of *Streptococcus viridans.*

The normal bone marrow of rabbits, examined in imprints stained by the May-Grünwald-Giemsa technic, contains relatively few plasma cells and plasmacytic reticulum cells. The bone marrow of rabbits allergic to *Streptococcus viridans* showed an increase in the number of plasmacytic reticulum cells and a slight increase in plasma cells. Within 20 minutes after an intravenous injection of the antigen into the allergic rabbits, the bone marrow showed a great increase in plasmacytic reticulum cells—large, immature, basophilic cells with eccentric leptochromatic nuclei. The shock dose of antigen, in addition to causing an increase in plasmacytic reticulum cells, induced the transformation of these cells into plasma cells. This transition was morphologically evident within 2 hours, and complete to large plasma cells in 5 hours. In 5 days, small plasma cells—small, mature, basophilic cells with an eccentric, pachychromatic nucleus. some of which bear evidence of degeneration—predominated in the bone marrow plasma cell picture.

In addition to the bone marrow changes, the spleens of our rabbits showed the "acute splenic tumor" described by Rich[6] as a reaction to an antigen bacterial or non-bacterial and probably related to antibody formation.

We conclude that the bone marrow plasma cell is a derivative of the plasmacytic reticulum cell. Since concurrently with the transformation of the plasmacytic reticulum cell to plasma cells the antibody titer of the blood rises (Clawson,[7] Rich[6]) there may be a relationship between these two phenomena.

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[6] Rich, A. R., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 1349.

[7] Clawson, B. J., *J. Inf. Dis.*, 1933, **53**, 157.

10124

Effect of Adrenalectomy During Gestation on the Size of the Adrenal Glands of Newborn Rats.

DWIGHT J. INGLE AND GERTRUDE T. FISHER. (Introduced by J. L. Bollman.)

From the Division of Experimental Medicine, The Mayo Foundation, Rochester, Minnesota.

The object of this study was to determine the extent to which the adrenal cortices of the fetal rat are responsive to a condition of adrenal insufficiency produced in the mother.

Albino rats of the Wistar strain, each weighing 180 to 190 g at the time of mating, were used. Of 10 animals, the adrenal glands were removed on the seventh day of pregnancy and of 15 animals, on the fourteenth day of pregnancy. Eighteen additional pregnant animals were not subjected to operation. On the twenty-first day of pregnancy the young were taken for weighing either immediately after delivery or by cesarean operation. The adrenal glands of the newborn were removed and carefully cleaned of fat and connective tissue. The glands from the animals of the same sex from each litter were combined and weighed in a closed bottle by one of us who was unaware of the identity of the mother. The procedure used in obtaining glands for weighing was carefully standardized and we believe that it was sufficiently accurate to insure reliability of the differences observed. The data are summarized in Table I.

TABLE I.
Data on Newborn Rats; Averages and Range.

Mother adrenalectomized	No. of litters	No. in litter		Body wt, g		Adrenal wt (each gland), mg	
		Male	Female	Male	Female	Male	Female
14th day of pregnancy	15	3.7 1-6	3.2 1-6	4.96 3.98-5.42	4.78 3.91-6.17	1.23 .94-1.44	1.18 .90-1.41
7th day of pregnancy	10	4.7 2-7	4.0 2-6	4.70 3.17-5.48	4.51 3.08-5.42	1.17 .92-1.53	1.13 .90-1.33
Normal	18	3.8 2-6	3.2 1-6	5.34 3.64-6.07	5.03 4.12-5.52	0.90 .72-1.19	0.82 .45-1.05

The average values for the weights of adrenal glands of the newborn from adrenalectomized mothers were significantly above normal in each group represented. All of the adrenal weights for the experimental animals were above the means for normals. Histologic comparison of the glands indicated that the differences in size were owing to an increase in the amount of cortical tissue in the glands from the experimental animals.

We have no proof that the glands of the fetus protected the mother against adrenal insufficiency. Mild symptoms of adrenal insufficiency were common among the pregnant mothers and we have observed, in other unpublished experiments, that a severe state of insufficiency is easily produced in these animals by mild stress, such as chilling, a 24-hour fast, and so on.

The mechanism by which the cortical hyperplasia is induced in the fetal adrenal gland was not studied in this experiment. There may be a hormonal stimulus arising from the pituitary body of the adrenalectomized mother or the stimulus may arise in the fetus itself in response to other abnormal changes in the fluids provided to the fetus by the mother.

10125

Capacity of Skeletal Muscle of Castrated Female Rats for Sustaining Work Output.

Dwight J. Ingle. (Introduced by J. L. Bollman.)

From the Divisions of Biochemistry and Experimental Medicine, The Mayo Foundation, Rochester, Minn.

In a previous experiment,[1] I have made a comparison of normal and castrated male rats in respect to the capacity of the gastrocnemius muscle for performing work when made to contract by faradic stimulation. In the present experiment, normal and castrated female rats were studied in a similar manner.

Albino female rats were closely matched into 10 pairs on the basis of weight and one animal in each pair was castrated during the second month following birth. Each rat was prepared for the work test when it reached a weight ranging from 185 to 195 g.

The animal was anesthetized with phenobarbital sodium, its gastrocnemius muscle was weighted with 100 g and was stimulated directly to contract 3 times per second until the muscle became "fatigued," or for 120 hours. The amount of work performed was recorded automatically. Each rat received 6 cc of a physiologic solution of saline by subcutaneous injection every 12 hours. A detailed description of the apparatus and procedure used has been presented.[2]

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[1] Ingle, D. J., unpublished data.

The data on averages and ranges of work for 10 pairs of rats are summarized in Table I. Inferiority as regards the ability of castrated female rats to sustain work of the gastrocnemius muscle was not observed in this experiment.

TABLE I.
Record of Work for 10 Normal and 10 Castrated Female Rats.

	Normal			Castrated		
		Range*			Range*	
Hr of work	Avg*	Minimum	Maximum	Avg*	Minimum	Maximum
0 to 24	61.2	41.1	82.9	60.7	32.1	70.9
24 to 48	31.3	9.2	52.6	28.6	6.5	42.1
48 to 72	33.6	20.6	42.7	36.3	16.7	50.3
72 to 96	39.3	28.7	49.9	45.6	22.4	61.3
96 to 120	32.4	0	50.5	42.8	21.8	60.9
Total period of work	197.8	147.2	240.5	214.0	99.7	259.1

* Expressed in kilogram-meters; error for relative values does not exceed 10%.

The results of this experiment are similar to those of my previous study on castrated male rats. They do not agree with the results of Miley,[3] who found that castrated female rats were deficient in their capacity for work.

The daily values for work in this experiment were occasionally higher than on the preceding day although the stimulation was continuous. This is a frequent observation on performance of work when studied under the experimental conditions used in this investigation.

10126

Effect of Cortin on Survival and Work Capacity of Rats after Removal of Intra-Abdominal Organs.

Dwight J. Ingle. (Introduced by Jesse L. Bollman.)

From the Division of Experimental Medicine, The Mayo Foundation, Rochester, Minnesota.

The site of action of cortin is unknown. In this study I have attempted to determine whether cortin will act after all the abdominal viscera have been removed from the rat. Adult male rats of the Wistar strain were used. The operations were carried out in two stages. In the first stage the vena cava was completely ligated between the points of entrance of the renal veins and those of the hepatic veins. Owing to the immediate circulatory changes induced by such a procedure, the adrenal glands are injured. Following this first stage of the operation death may occur in 40 to 60% of cases. Because of the possible relation between the adrenal gland and survival, I treated all of the animals with 1 cc of cortin daily in their drinking water for 5 days following operation. The mortality rate was reduced to less than 10% by this procedure.

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[2] Heron, W. T., Hales, W. M., and Ingle, D. J., *Am. J. Physiol.*, 1934, **110**, 357.

[3] Miley, H. H., *Am. J. Physiol.*, 1927, **82**, 7.

Following a minimal period of delay of 21 days during which time an adequate collateral circulation was established the animals were closely matched into pairs on the basis of weight and all of the intestines, liver, kidneys and adrenal glands were removed from the abdominal cavity. Ether was used for anesthesia.

In Experiment 1, each of 10 pairs of animals was given 1 cc of a 20% solution of glucose immediately following operation and at each succeeding interval of 2 hours. One animal of each pair was given 0.5 cc of cortin (each cubic centimeter of cortin represented 75 g of whole gland) immediately following operation and at each succeeding interval of 2 hours. Of each pair one animal was used as a control; it received similar amounts of a physiologic saline solution at intervals of 2 hours. The animals which received injections of cortin survived for an average of 28 hours with a range of 20 to 32 hours. The animals which were not treated with cortin survived for an average of 17 hours with a range of 8 to 26 hours.

In Experiment 2, ten pairs of animals, all the abdominal viscera of which had been removed, were anesthetized with phenobarbital sodium and the gastrocnemius muscle was stimulated to lift 100 g three times per second. The injections were made in the same manner as in Experiment 1. The work done was registered automatically. The details of the methods used have been described.[1]

The initial rates of work among eviscerated rats were similar to those among the animals not operated on and, during the first 3 hours of work, the height of muscular contraction was well maintained among all of the animals. Those animals which received cortin worked for longer periods of time and performed more work before insufficiency occurred than did the controls. The data of Experiment 2 are presented in Table I.

It has not been generally recognized that an animal may be made

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[1] Heron, W. T., Hales, W. M., and Ingle, D. J., *Am. J. Physiol.*, 1934, **110**, 357.

TABLE I.
Work of Pairs of Completely Eviscerated Rats.

Body wt, g		Initial rate of work*		Total work		Hr work*	
Cortin	No cortin	Cortin	No cortin	Cortin	No cortin	Cortin	No cortin
270	270	15	15	4270	3774	13.0	10.0
215	215	12	15	7296	2545	18.0	6.5
290	295	17	17	6674	5785	16.0	9.5
235	230	17	15	7703	3577	14.0	7.5
280	280	15	18	6137	4505	12.5	7.0
320	300	21	18	7788	3953	11.0	4.5
225	225	17	16	7320	2342	19.0	5.0
240	240	15	17	4075	2848	12.5	6.5
240	245	13	17	7014	2742	11.0	5.0
380	380	22	25	7754	2543	10.5	5.5

*Expressed as recorder revolutions. Each revolution is the equivalent of approximately 400 g-cm of work. The error for relative values does not exceed 10%.

to manifest adrenal insufficiency within a few hours after removal of the adrenal glands. It is true that when the rat is adrenalectomized with a minimum of trauma and is maintained under conditions optimal for survival it may live indefinitely without treatment with cortin. On the other hand, when the adrenalectomized animal is made to undergo severe stress, insufficiency may be induced very rapidly unless large amounts of cortin are administered frequently.[2] This has been observed in my own experiments and by many other investigators.

It is evident that cortin can act in the animal organism, that is, it can maintain life, after all the organs of the peritoneal cavity have been removed. This cannot be interpreted to mean that cortin does not influence these organs when they are present, because there is a large amount of experimental data which indicates that the functions of the kidneys and of the liver are altered by either a deficiency or an excess of cortin in the body.[3] The results of the experiments reported herein support the hypothesis that cortin does not have a specific site of action in the body which is essential for all of its effects. It is of additional interest that a normal rate of muscular work can be sustained for several hours by an animal treated with glucose, from which the liver and pancreas have been removed.

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[2] Ingle, D. J., and Kendall, E. C., *Am. J. Physiol.*, 1936, **117**, 200.

[3] Ingle, D. J., Nilson, H. W., and Kendall, E. C., *Am. J. Physiol.*, 1937, **118**, 302.

10127

Subcutaneous Administration of Cortin Compounds in Solid Form to the Rat.

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Numerous substances having a low solubility in the body fluids have been administered experimentally by implanting them in solid form within the body tissues. We have administered to rats solid forms of extracts of adrenal cortex and 3 pure crystalline compounds which are identified in the literature as A, B, and E.[1]

In Experiment 1, ten male rats having a range in body weight of from 100 to 110 g were matched into pairs and were all adrenalectomized in single-stage aseptic operations. At the time of adrenalectomy a single crystal of compound E which did not exceed 2 mg in weight was implanted subcutaneously into one animal of each pair. The 5 untreated control animals developed symptoms of adrenal insufficiency and died at intervals of 5, 6, 10, 10, and 12 days after operation. The animals which had received the single implants of compound E died at intervals of 15, 20, 23, 23, and 28 days after operation.

For Experiment 2, a total extract of cortin was prepared for implantation. Compounds A, B, E, and some biologically inactive compounds were removed by fractionation. A sample of 330 cc was evaporated to dryness in a vacuum at 40°C. It was redissolved in absolute alcohol and again dried. This was repeated with acetone and the residue of 105 mg was dissolved in alcohol with 400 mg of cholesterol. The mixture was dried by warming in a stream of air. The residue was moistened with alcohol and thoroughly mixed. It was dried in a vacuum desiccator, again moistened with alcohol and compressed into pellets. Twelve male rats having body weights of from 60 to 70 g were closely matched into pairs and all were completely adrenalectomized. A single pellet was implanted under the skin of the dorsal neck region in one animal of each pair. The control animals developed symptoms of adrenal insufficiency and died at intervals of 3, 5, 5, 8, and 10 days after operation. Those animals which had received the pellets grew in a normal manner. Four weeks after operation these animals were killed and the pellets removed, dried and weighed. The data for these animals are presented in Table I.

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[1] Mason, H. L., Hoehn, W. M., McKenzie, B. F., and Kendall, E. C., *J. Biol. Chem.*, 1937, **120**, 719.

TABLE I.
Data on Adrenalectomized Rats Maintained by Subcutaneous Pellets Containing Cortin.

Experimental condition	Body weight, g		Weight of pellet, mg	
	Initial	Final	Initial	Final
Cortin pellets *in situ* 28 days	68	184	52.9	43.0
	69	172	45.2	40.0
	70	196	39.5	31.0
	70	170	44.0	33.5
	70	177	49.8	39.5
Compound B pellets *in situ* 21 days	68	100	60.2	58.0
	65	135	55.3	51.5
	60	124	55.2	53.0
	68	116	44.5	42.0
	60	121	46.3	43.5

For Experiment 3, compound B was prepared for implantation. One hundred milligrams of B and 400 mg of cholesterol were dissolved together in chloroform. The additional procedures for preparing the pellet were identical with those following the mixture of cholesterol and the total cortin extract in Experiment 2. Twelve rats weighing 60 to 70 g were matched into pairs and all of the animals were completely adrenalectomized. At the time of operation a single pellet was implanted subcutaneously in the dorsal neck region. The 6 untreated rats died at intervals of 5, 6, 6, 6, 7, and 7 days after operation. Those animals which received pellets grew in a normal manner. They were killed 3 weeks after operation and the pellets were removed, dried and weighed. The data are presented in Table I.

Atrophy of the adrenal cortex and of the thymus[2] occurs after the oral administration of large amounts of cortin to normal male rats. In Experiment 4, atrophy of the adrenal cortex was produced by the administration of cortin in solid form. Normal male rats each having an initial weight of 180 g were used. Among such animals the range in weight of pairs of adrenal glands is from 22 to 32 mg with an average of 27 mg. The range of thymus weights is 280 to 540 mg with an average of 410 mg. Each rat was killed one week after the implantation of the pellets. The adrenal glands, the thymus and the pellets were removed and weighed. Twelve milligrams of pure compound A lost 7.5 mg weight. The adrenal glands weighed

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[2] Ingle, D. J., Higgins, G. M., and Kendall, E. C., *Anat. Rec.*, 1938, **71**, 363.

14.5 mg and the thymus 101 mg. Twelve milligrams of pure compound E lost 6.5 mg weight. The adrenal glands weighed 19 mg and the thymus 188 mg. Pellets containing 20% cortin (containing all of the compound E and some of compounds A and B which were present in the original total extract) and 80% cholesterol were prepared as in Experiment 2. These pellets were implanted into 4 rats. One pellet weighing 54 mg lost 6.5 mg during one week. The adrenals weighed 30 mg and the thymus 433 mg. Two pellets having a total weight of 95.5 mg lost 26.5 mg in one week. The adrenals weighed 27 mg and the thymus 425 mg. Four pellets weighing 220 mg lost 37 mg in one week. The adrenals weighed 17 mg and the thymus 68 mg. Six pellets having a total weight of 302 mg lost 52 mg in one week. The adrenals weighed 15 mg and the thymus 63 mg.

Our data suggest that cortin compounds may be more efficiently utilized when administered to the rat in this manner than when given by the other usual methods. However, there may be appreciable errors in our evaluations of the extent of loss of cortin material by weighing. There may have been a differential absorption of the cortin compounds and of the cholesterol from the pellets and there may have been a cellular infiltration of the pellet which masked the true weight loss to some degree.

10128

Phagocytosis of Leishman-Donovan Bodies by Leukemic Blood Cells.

CHIA-TUNG TENG AND HUEI-LAN CHUNG. (Introduced by F. R. Dieuaide.)

From Peiping Union Medical College.

Since kala-azar produces characteristically a marked leukopenia in patients, it seemed interesting to know what effect this infection might produce on the blood picture of patients with chronic myelogenous leukemia. As we had no opportunity to observe this in patients, we thought it worthwhile to study the reaction of leukemic blood cells *in vitro* on Leishman-Donovan bodies, *i. e.,* their phagocytic activity. Incidentally it is also of interest to find out what the relative phagocytic activity of various types of leukemic blood cells is, especially in view of the fact that leukemic patients in general do not resist infections well in spite of the increased number of circulating leukocytes.

The spleen, aseptically removed from a Chinese hamster *(Cricetulus grieseus)* heavily infected with *Leishmania donovani,* was emulsified in 5 cc sterile physiologic saline. The emulsion was centrifuged for a few minutes at a low speed (500 rpm), and the sediment discarded. The supernatant suspension of Leishman-Donovan (L.D.) bodies was then ready for use. Twenty cc of leukemic blood were obtained from a cubital vein of a patient* with chronic myelogenous leukemia, 1 cc of 3.8% sodium citrate being used to prevent coagulation. The blood was allowed to sediment spontaneously at a temperature of 36°C in an incubator. The plasma was removed and discarded, the middle "buffy" layer was separated from the red cells and used for the experiment. A small drop of this leukocytic cream was mixed with an equal drop of the L.D. suspension on each of 12 pieces of mica cover-slips, mounted separately on 12 hollow-ground glass slides as hanging drops, sealed with sterile vaseline and incubated in an incubator at 36°C. The hanging drops were examined after various periods of incubation, and ordinary smears were made and stained with Wright stain. A differential count was then made on these smears. A summarized result is as shown in Table I.

From the table it is clear that neutrophils from the leukemic patient have very strong phagocytic activity on L.D. bodies, metamyelocytes have less and myelocytes still less activity. On the other hand none of the lymphocytes, basophils, basophilic and eosinophilic myelocytes, and myeloblasts showed such an activity, while no monocytes were seen. Eosinophils only occasionally phagocytosed the parasites. It must, however, be pointed out that the number of such cells observed is too few and the presence of basophilic and to some extent eosinophilic granules made the identification of L.D. bodies, if any, in these particular cells rather difficult.

Besides the stained smears, supravital preparations were also made from the mixtures of the L.D. suspension and the leukocytic cream, and examined in a warm chamber. Active phagocytosis was found as early as within 5 to 10 minutes. The relative activity of the various types of cells was same as observed in the Wright-stained smears. We also found that the cells which phagocytosed L.D. bodies tended to degenerate and disintegrate more rapidly than those which did not.

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* The patient was a Chinese male of 34, having symptoms of the disease of 7 months' duration. The total white blood cell count at the time of the experiment was 164,000 per mm^3. He was later treated with a course of deep X-ray irradiation over the spleen with prompt remission.

TABLE I.

Phagocytosis of Leishman-Donovan Bodies by Various Types of Leukemic Blood Cells.

No. L.D. in each cell	Phagocytosis												No phago-cytosis		Total	
	1		2		3		4		4+		Total					
Cells	No. Cells	%	No. Cells	%	No. Cells	%	No. Cells	%	No. Cells	%	No. Cells	%	No. Cells	%	No. Cells	%
Lymphocyte	0	0	0	0	0	0	0	0	0	0	0	0	8	100	8	0.8
Monocyte	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Neutrophil	57	27.2	47	22.5	33	15.8	18	8.6	15	7.1	170	81.3	39	18.7	209	20.9
Basophil	0	0	0	0	0	0	0	0	0	0	0	0	27	100	27	2.7
Eosinophil*	0	0	0	0	0	0	0	0	0	0	0	0	5	100	5	0.5
N. Meta-myelocyte	20	22.7	7	8.0	3	3.4	3	3.4	1	1.1	34	38.6	54	61.4	88	8.8
N. Myelocyte	18	2.9	12	1.9	5	0.8	2	0.3	3	0.5	40	6.5	578	93.5	618	61.8
B. Myelocyte	0	0	0	0	0	0	0	0	0	0	0	0	4	100	4	0.4
E. Myelocyte	0	0	0	0	0	0	0	0	0	0	0	0	30	100	30	3.0
Myeloblast	0	0	0	0	0	0	0	0	0	0	0	0	11	100	11	1.1
														Total	1,000	100

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*After examining more cells, we found that eosinophils also occasionally phagocytose L.D. bodies.

The same experiments were repeated, using the blood of another patient† with chronic myelogenous leukemia and also that of a normal individual, and very similar results were obtained. (Lymphocytes and monocytes in normal blood were never seen to show any phagocytic activity, but the number of the latter cells encountered was too small to permit a conclusion).

We also tested the phagocytic activity of various types of leukemic blood cells on *pneumococcus type I* and *streptococcus hemolyticus* and our findings were in agreement with those reported by Strumia and Boerner.[1]

Conclusion. Neutrophilic leukocytes beyond the myeloblastic stage, and occasionally the eosinophils, from the blood of patients with chronic myelogenous leukemia phagocytize L.D. bodies *in vitro.*

10129

Intracutaneous Immunization of Rabbits with Photodynamically Inactivated Type I Pneumococcus.

T. T'UNG. (Introduced by S. H. Zia.)

From the Department of Bacteriology and Immunology, Peiping Union Medical College, Peiping.

Julianelle,[1] employing the endermal route of immunization of rabbits with heat-killed type I pneumococcus, could not demonstrate any significant agglutinative or precipitative response to the homologous organism and specific soluble substance respectively. He ascribed this failure to the fact that the pneumococcus undergoes disintegration in the skin of rabbits and this results in the destruction of the type-specific antigen. The rapid extracellular digestion by tissue-ferments of heat-killed pneumococci when injected into the skin, as demonstrated by Dubos and Macleod,[2] lends some support to this idea. In view of the fact that the pneumococcus inactivated by the photodynamic action of methylene blue is much more resistant to autolysis than that treated with formalin or heat,[3] it was considered of interest to determine whether or not type I pneumococcus so treated could serve as an effective antigen when injected into the skin. The present investigation was therefore undertaken to study the comparative efficacy of methylene-blue-treated vaccine with those prepared by formalin or heat in immunizing rabbits by the endermal route.

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† The second patient was a Chinese male of 28, who had been suffering from the disease for one year and 8 months. The total leukocyte count at the time of the experiment was 179,000 per mm^3. He was also subsequently treated with deep X-ray irradiation which brought about a satisfactory remission.

[1] Strumia, M. M., and Boerner, F., *Am. J. Path.*, 1937, **13**, 335.

[1] Julianelle, L. S., *J. Exp. Med.*, 1930, **51**, 441.

[2] Dubos, R. J., and Macleod, C. M., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 696.

Pneumococcus type I used for this experiment was one of high virulence; 0.5×10^{-9} cc of an 18-hour broth culture killed white mice regularly. The photodynamically inactivated and the formolized vaccines were prepared according to methods described previously,[3] while the heat-killed vaccine was prepared by Julianelle's method.[1] The method of immunization was essentially the same as Julianelle's, except that daily rather than weekly inoculation was adopted. Each animal was given 6 consecutive injections of 0.2 cc each followed by a week of rest. The same course of immunization was repeated once and bleeding was done 10 days after the last injection. The amount of vaccine given in 4 weeks was more or less equal to that employed by Julianelle in 3 months. For agglutination and precipitation homologous organisms and soluble specific substance were used.

After 2 courses of immunization it was found that of a total of 10 rabbits immunized with photodynamically inactivated vaccine, the serum of one had a complete-agglutination titer of 1-128; three, 1-64; four, 1-32; and two, 1-16. On the other hand, out of a total of 6 animals immunized with formolized vaccine, the sera of 2 had a "complete" titer of 1-32; two, 1-16; and two, negative. Among 5 animals immunized with heat-killed vaccine, the serum of only one yielded a complete titer of 1-32; two, 1-16; one, 1-8; and one, negative. Precipitative reactions with soluble specific substance also showed higher titers of sera from animals immunized with photodynamically inactivated vaccine. Four sera of a higher agglutinative titer reacted with this polysaccharide up to the dilution of 1-500,000 and the rest 1-100,000. Two of the rabbits immunized with formolized vaccine had precipitative titers of 1-100,000 and another two, 1-10,000. The 2 sera that did not agglutinate also did not give any precipitative reaction. Sera of animals immunized with heat-killed vaccine had low precipitative titers, namely 1-50,000 in one, and 1-10,000 in two. Precipitin could not be demonstrated in the serum of the rabbit having the lowest agglutinative titer or of the one which gave no agglutination. Complement-fixation tests were also done on all sera and it was found that in general, the titer was about 10 to 20 times higher than that obtained by precipitation.

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[3] T'ung, T., *Chinese Med. J.*, Supplement II, 1938, 291.

Only sera having the higher agglutinative and precipitative titers were tested for passive protection of white mice. It was found that whereas 0.2 cc of each of the 4 sera prepared with photodynamically inactivated vaccine protected mice against 10,000 to 100,000 MLD, the same amount of 3 sera from each of the other 2 series of animals protected mice against only 1000 MLD.

The superiority of the photodynamically inactivated vaccine over those treated either with formalin or by heat in the immunization of rabbits by the intracutaneous method was further substantiated by the fact that rabbits which had failed to respond to formolized or heat-killed vaccine yielded significantly high agglutinative and precipitative titers when these animals were given another 2 courses of immunization with photodynamically inactivated vaccine by the same route.

The results of these experiments offer further proof that photosensitized pneumococcal vaccine is more efficacious than vaccines prepared with formalin or heat.

10130

Antigenic Activity of Polysaccharides of *B. rhinoscleromatis.*

Sam C. Wong and T. J. Kurotchkin.

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Polysaccharide obtained from *B. rhinoscleromatis* through extraction of dried bacterial cells in weak acetic acid solution provokes in immunized rabbits the formation of complement-fixing antibodies and induces in guinea pigs active sensitization. In this experiment an attempt was made to demonstrate that the antigenicity of the polysaccharide of this bacterial species is analogous to that of the whole bacterial cell. It seemed also of interest to compare the immunological properties of this polysaccharide with that prepared by the method in which the treatment of the bacteria by heat was avoided.

A stock culture of *B. rhinoscleromatis* was passed through mice 3 times until its agar culture acquired markedly slimy appearance. The first type of polysaccharide was prepared in accordance with the method previously reported.[1] The second type of polysaccharide was obtained from the same microörganism by spontaneous autolysis of bacterial suspension in distilled water. Polysaccharides prepared in this manner showed none of the usual protein reaction, the amount of nitrogen in both types of substance being equal to 0.24%. Immunological reactivity of the polysaccharides was determined by testing them against immune rabbit serum prepared by immunization of these animals with formol killed culture of *B. rhinoscleromatis*. It was found that both substances reacted equally well giving positive precipitation and complement-fixation tests in concentration of 1:6,000,000 and 1:64,000,000 respectively. Cross absorption tests showed that the polysaccharide prepared by autolysis of bacterial bodies removed from anti-bacterial serum all precipitin, whereas, the serum absorbed with the polysaccharide prepared by extraction with acetic acid still gave precipitin reaction to the titer of 1:500,000 when tested against the former type of substance. This indicated that the polysaccharide obtained by autolysis is more complex than that obtained by acid extraction.

Eight rabbits were immunized with each type of the substances. Each animal received a total of 9 injections, each of which contained one mg of the substance dissolved in one cc of normal saline. The injections were given on 3 successive days and followed by 4 days of rest. The examination of the sera of all these animals showed that precipitin reaction regularly occurred when 1:1000 concentration of either type of polysaccharides was used. With sera from 2 rabbits a strongly positive test was obtained when 1:10,000 dilutions of the polysaccharides were used. Complement fixation occurred in dilutions from 1:1000 to 1:100,000 of both substances tested against anti-polysaccharide sera in 1:5 dilution. Agglutination reaction performed with a suspension of the rough form of *B. rhinoscleromatis* derived from the smooth culture showed a titer equal to 1:128, whereas the titer of 1:8 was observed when a decapsulated smooth form was used for agglutination. An attempt was made to sensitize guinea pigs with the antipolysaccharide sera giving the best precipitin test. Ten guinea pigs weighing between 150 to 200 g, each received intraperitoneally 3 cc of serum per animal. Twenty-four hours later the animals were tested for hypersensitivity by intravenous injections of 5 mg of either type of the polysaccharides. All but one animal gave negative result. One guinea pig injected with the polysaccharide prepared by acid extraction developed symptoms of typical non-fatal anaphylactic shock.

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[1] Wong, S. C., Proc. Soc. Exp. Biol. and Med., 1938, **38**, 107.

Recent evidence[2, 3] seems to indicate that antigenic polysaccharides are heat labile. This conclusion is not borne out by our observations. It may seem obvious that the antigenic component of the polysaccharide prepared by acid extraction is heat stable since the use of heat is involved during the process of extraction and final drying of the substance. The heat stability of the antigenic component of the polysaccharide prepared by spontaneous autolysis of bacterial cells is proved by the experiment in which 0.1% solution of the polysaccharide was heated in boiling water either alone or in the presence of 0.5% of potassium hydroxide. Immunization of rabbits with fractions prepared in this manner showed that only the alkali-treated polysaccharide failed to immunize.

The above findings indicate that 2 polysaccharides identical in immunological properties but differing in their absorptive power can be prepared from the cultures of *B. rhinoscleromatis,* and that the rôle played by acetic acid in the process of extraction is merely one of preserving the antigenic property of the substance.

10131 P

Isolation of Typhus Fever Virus from House Rats in Peiping.

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In our previous studies[1] on the typhus fever virus isolated from local patients, two distinct types were encountered, one simulating the classical epidemic type and the other, the endemic variety of the New World. At that time it was suggested that these two types of typhus fever might simultaneously exist in Peiping, though the popular impression hitherto was that typhus fever seen here was of the epidemic form. Recently Chung and Chang[2] studied a strain of virus isolated from a patient and identified it to be *Rickettsia prowazekii,* thus confirming the existence of the classical epidemic type. It occurred to us that if our contention regarding the coexistence of endemic typhus fever should be correct it would be of considerable interest to determine if local rats served as reservoir host, a fact already found to be true in the United States, Mexico, and countries surrounding the Mediterranean Sea[3] and in Manchuria.[4] This communication reports the result of study on this phase of the typhus problem.

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[2] Landsteiner, K., and Chase, M. W., *Ann. Rev. Bioch.*, 1937, **6**, 626.

[3] Haworth, W. N., and Hirst, E. L., *Ibid.*, 1937, **6**, 104.

[1] Wu, C. J., and Zia, S. H., *Chin. M. J.*, 1938, Suppl. II, 221.

[2] Chung, H. L., and Chang, J. H. M., *Chin. M. J.*, 1938, **53**, 513.

The common house rats were collected from different households in the city, mostly from the district in which the College is located. Lots of 2 or 3 rats were killed with ether and their brains removed aseptically and emulsified in physiological saline. Half of the total suspension was injected intraperitoneally into one guinea pig. According to the usual technic employed in experimental typhus studies the body temperature of inoculated pigs was taken daily, and passage in guinea pigs was carried out with brain or blood-brain mixture of those animals showing definite elevation of temperature over 40°C. All surviving guinea pigs were kept under observation for one month and were then discarded if showing no fever.

We have studied in this manner 139 house rats. From one lot of 3 rats we obtained a strain of typhus fever virus on which a few preliminary studies have been made and further studies are still in progress. These rats were caught on March 25, 1938, from a meat shop. The guinea pig which was inoculated with the brain emulsion developed fever on the ninth day and typical, though not marked scrotal swelling on the tenth day when the animal was sacrificed for serial transfer. We have now carried the virus in guinea pigs for 7 generations. The infection is manifested by fever after an incubation period of 7 to 8 days and also invariably by scrotal swelling in the male guinea pigs. From tunica scrapings of one animal each of second and fifth generation passages typical Rickettsia bodies were seen on smears and grown in both Maitland and Zinsser's tissue agar cultures.[5] Cultures of the tunica tissues on the ordinary media for bacteria have been sterile. Blood sera from 3 third generation pigs have been tested for the presence of agglutinins for Weigl vaccine and found to be positive to the titer of 1:40 in 2 specimens and 1:10 in the third. Two of these animals showed complete protection against the Mexican virus while the third animal died a few days after the test inoculation. The virus was also infective for the local rat-mole[6] giving rise to fever and positive Weil-Felix reaction.

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[3] Zinsser, H., *Am. J. Hyg.*, 1937, **25**, 430.

[4] Kodama, M., Takahashi, G., and Kohno, M., *Kitasato Arch. Exp. Med.*, 1932, **9**, 87.

[5] Zinsser, H., Wei, H., and Fitzpatrick, F., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 285.

[6] Zia, S. H., and Hu, C. H., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 26.

From the results so far obtained it seems justifiable to conclude that we are dealing with a strain of typhus fever virus isolated for the first time from local house rats.

10132

Influence of Genetic Relationship on the Success of Homeoplastic Transplants of Adrenal Glands in Albino Rats.

DWIGHT J. INGLE AND GEORGE M. HIGGINS. (Introduced by J. L. Bollman.)

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It has been demonstrated in studies by Wyman and tum Suden[1] and by us[2] (with Nilson) that homeoplastic transplants of adrenal glands may be successful when carried out between adult rats of inbred strains. This is in contrast to the consistent failure of adrenal transplants to become established as functional grafts when carried out between animals of different strains.

The present report is concerned with a further investigation of the problem of homeoplastic transplants of adrenal glands in a Wistar strain of rats which had not been inbred. Three series of experiments were carried out in which the adrenal glands were exchanged between 20 pairs of siblings, 20 pairs of first cousins, and 20 pairs of rats more remotely related than second cousins.

The animals used were all adult female rats of an albino strain. The animals were matched into pairs according to body weight and were operated under ether anesthesia employing aseptic technic. The adrenal glands were removed from one animal through a lumbar incision and sutured to the ovaries of the other animal of the pair. The adrenals of the second animal were removed and likewise sutured to the ovaries of the first. All animals were given maintenance doses of cortin for 10 days after the operation. A standard diet was fed which contained 0.24% sodium and 1% potassium. All grafts were removed surgically from those animals which survived for 120 days. These animals were again given maintenance doses of cortin for 10 days following this second operation and their subsequent survival periods were noted. After withdrawal of cortin all but 2 of the animals died within a period of 25 days. The transplanted glands of these 2 animals were found to be degenerated. One of them was found to have accessory cortical tissue at necropsy.

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[1] Wyman, L. C., and tum Suden, C., *Endocrinol.*, 1937, **21**, 523.

[2] Ingle, D. J., Higgins, G. M., and Nilson, H. W., *Am. J. Physiol.*, 1938, **121**, 650.

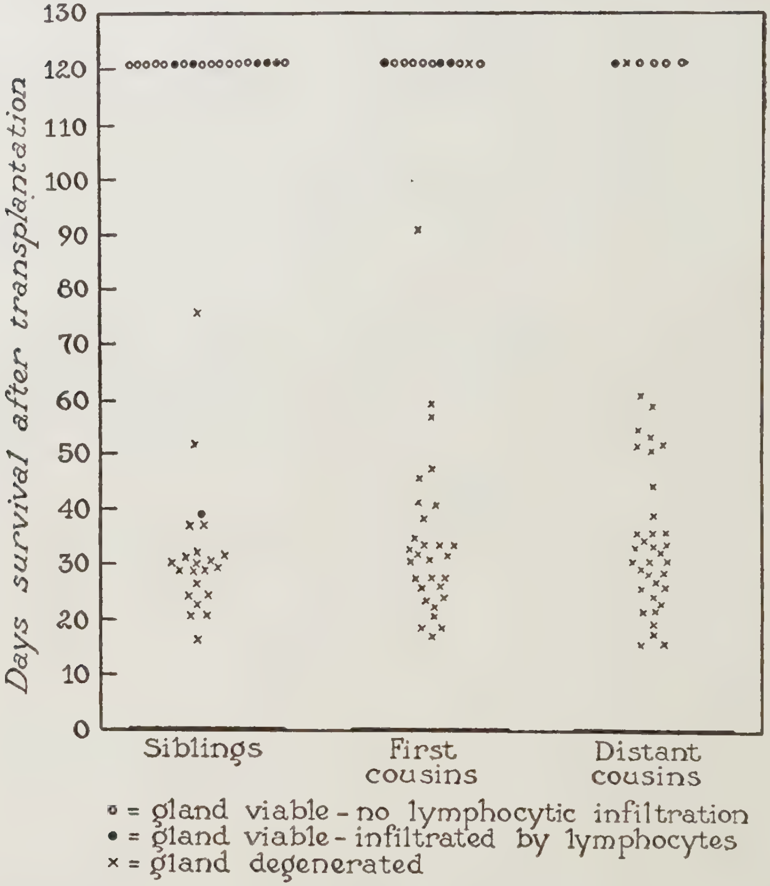

FIG. 1.

Length of survival of homeoplastic transplants of adrenal glands in a strain of albino rats.

The data on survival and on the condition of the transplanted tissue are summarized in Fig. 1. A marked difference in the incidence of success is noted. The incidence of successful grafts was much greater among siblings, less so among first cousins and least so among distant cousins.

10133 P

Temporary Agglutinability of Red Blood Cells.

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As a result of studies on atypical isoagglutination it was concluded that the atypical feature is an unusual isoagglutinin acting at room temperature (20°-25°) on cells of the same blood group, or on cells of group O. These atypical agglutinins which occur in about 3% of normal individuals, possess a distinct specificity, the great majority describing the subgroups A^1 and A^2, or the agglutinable substance known as P. Landsteiner and Levine[1] emphasized that the atypical property is a peculiarity, not of the blood cell, which could be readily grouped, but of the agglutinin in the serum. Consequently these atypical reactions do not interfere with the scheme of the 4 blood groups.

In the blood to be reported, however, the results indicate that in this instance (a patient of group O) the exceptional property is the agglutinability of the cell which could be demonstrated by its sensitivity to an agglutinin present in about 15% of the sera, irrespective of the blood group. This peculiar property of the cell could be demonstrated during the illness of the patient, but not after recovery. From this it was concluded that the agglutinable property was induced by the specific nature of the illness (soluble products of pneumococcus type I) or by the therapeutic substances employed.

Patient A. F., age 4, developed measles on March 17, 1938, and on the following day was admitted to the Willard Parker Hospital of New York City. The patient also showed signs of otitis media, bronchopneumonia, and later also fluid in the right pleural cavity from which pneumococcus type I was cultured. The same organism was also recovered from a pharyngeal swab and from blood cultures. Within the course of 2 days, the patient received 100,000 units of horse antipneumococcal serum (Types I and II); sulfanilamide was also given. The temperature ranged between 101° and 104°. On March 31 a thoracotomy was performed.

On April 11, the patient was somewhat improved, but still running a high temperature. At this time the patient's blood was

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* Aided by a grant from the Blood Transfusion Betterment Association of New York City.

[1] Landsteiner, K., and Levine, Philip, *J. Immunol.*, 1929, **17**, 1.

grouped and found to belong to group O. A donor sent by the Blood Transfusion Betterment Association was found compatible in the more important cross-test, *i. e.,* the patient's serum failed to act on the cells of the prospective donor. However, on testing the serum of the donor and cells of the patient, distinct agglutination was observed. These specimens were then referred to the Blood Transfusion Betterment Association for further study.

On repeating the tests the observation made was confirmed. It could be shown that the serum of the donor agglutinated distinctly, but weakly, the cells of the patient (group O) both at room temperature (25°C) and more distinctly at 20°. When the tests were put up at 37°, no agglutination was observed. On testing the prospective donor's serum with the numerous O cells at 20°, not one was found to be sensitive to this agglutinin. This in itself was very unusual, since it was assumed from previous studies[1] that when atypical agglutinin was found, it never reacted on one blood only but invariably on several bloods. The second unusual feature was that the agglutinable property in the cells could be detected by the demonstration of the agglutinin in the sera of about 15% of all individuals irrespective of the blood group. Consequently the situation is the reverse of what was previously considered characteristic of atypical reactions, *i. e.,* rare incidence of the atypical agglutinins and wide distribution of the agglutinable factor in contrast to a high incidence of the agglutinin and an agglutinable substance specific for one blood only.

The agglutinin was found to be specifically absorbable by the sensitive cells of the patient. In view of the specific nature of the reaction, an attempt was made to determine if the factor is hereditary. This was found not to be the case, since the cells of neither the father nor the mother were sensitive to the specific agglutinin either in the direct test, or in the absorption test. Thus the agglutinable factor seems to be an induced and not a constitutional property, and this is supported by the fact that the agglutinability of the cells disappeared after the recovery of the patient.

From one case it is impossible to establish a correct explanation of this hitherto undescribed phenomenon, but as a working hypothesis it is conceivable that disease products, horse serum, sulfanilamide, or possibly other medications, by the specific absorption to the red cells or some other mechanism could so alter the blood as to render them susceptible to the action of a physiologically occurring agglutinin of wide distribution. This hypothesis is compatible with the fact that the reaction was still observed in cells which were washed several times in a large volume of saline and also that the property could no longer be found in the red cells 4 months later, after which interval the original sensitive red cells presumably had been displaced from the circulation by a new supply.

The authors are indebted to Drs. Dolgopol and Kannerstein of the Willard Parker Hospital for the privilege of studying this blood.

10134

Correlation Between Blood Ascorbic Acid and the Dichlorophenolindophenol Intradermal Test.

WALTER W. JETTER. (Introduced by Guy E. Youngburg.)

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Rotter[1] suggested as a skin test the intradermal injection of a dilute solution of dichlorophenolindophenol as an index of the vitamin C saturation of the body tissues. Working on guinea pigs, he noted that the color of the dye injected intradermally decolorized much more rapidly in the normal than in the scorbutic animals. Similar results were also obtained in human beings. He concluded that if decolorization of the dye occurred in less than 5 minutes vitamin C "saturation" of the body tissues was present, whereas a decolorization time of 5-10 minutes was considered normal and more than 10 minutes indicated hypovitaminosis. Corroborative results were also obtained, in a study of 103 cases, by Portnoy and Wilkinson,[2] who, however, recognized that the decolorization of the dichlorophenolindophenol solution might not necessarily be due to specific reduction by ascorbic acid and that other substances such as glutathione might also have some part in the reaction. The advantages of a skin test as a rapid method for determining the degree of saturation of vitamin C in the body tissues are obvious. For this reason, it was thought advisable to evaluate the intradermal test on a series of 50 patients.

A series of 50 adult female patients, in all of whom active tuberculosis was present, were selected. All were on a diet rich in vitamin C. The technic employed was as follows: the fasting ascorbic acid content of whole blood was determined using the method of

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[1] Rotter, H., *Wein. Klin. Wochenschr.*, 1938, **51**, 205.

[2] Portnoy, B., and Wilkinson, J. F., *Brit. Med. J.*, 1938, **1**, 328.

Farmer and Abt,[3] the analyses being performed within 2 hours after the taking of the sample. At the same time, the intradermal test was performed, according to the technic outlined by Rotter. Three intradermal wheals, approximately 2-3 mm in diameter, were made on the anterior surface of the forearms and the time required for the disappearance of the blue color noted. The dichlorophenolindophenol was freshly prepared and was approximately n/500 in strength, being sterilized by passage through a Seitz filter.

TABLE I.
Decolorization Time in Minutes of Dichlorophenolindophenol Injected Intradermally.

Mg % blood ascorbic acid	No. of cases	1-2	2-3	3-4	4-5	5-6	6-7	7-8	8-9	9-10
.40- .49	4	—	2	—	1	1	—	—	—	—
.50- .59	8	—	1	4	2	—	—	—	1	—
.60- .69	9	—	1	2	1	4	1	—	—	—
.70- .79	4	—	2	—	1	1	—	—	—	—
.80- .89	7	—	1	2	1	1	2	—	—	—
.90- .99	7	—	—	2	1	3	—	1	—	—
1.00-1.09	4	—	—	—	1	2	1	—	—	—
1.10-1.19	3	—	—	—	1	—	1	—	—	1
1.20-1.29	1	—	—	—	—	1	—	—	—	—
1.30-1.39	1	—	—	—	—	—	1	—	—	—
Above 1.39	2	—	—	—	1	—	—	1	—	—
Total	50	—	7	10	10	13	6	2	1	1

In Table I it will be noted that the blood ascorbic acid content of the 50 cases was below 1.39 mg % in all but 2 instances, the majority of the cases (35) falling into a bracket from 0.50 to 1.00 mg % ascorbic acid. Four cases were between 0.40 and 0.49 mg % while 7 cases were in a group between 1.10 and 1.19 mg %.

In 46 of the cases, decolorization of the dye injected intradermally occurred in from 2 to 7 minutes, while in 33 of the 46 cases decolorization occurred in from 3 to 6 minutes. The other 4 cases required from 7 to 10 minutes. No cases were encountered requiring less than 2 or more than 10 minutes. As seen in the table, no correlation could be made between the ascorbic acid content of the blood and the decolorization time of the intradermal test. Isolated examples illustrating further the discrepancy between the chemical and the intradermal test are: a moribund patient with a blood ascorbic acid content of 0.42 mg % showed by the intradermal test decolorization times of 1:30, 1:35, 1:55, 2:10, and 2:40 minutes; 2 cases with blood ascorbic levels above 1.39 mg % had an average decolorization time of 4-5 and 7-8 minutes respectively.

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[3] Farmer, C. J., and Abt, A. F., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 1625.

If one accepts values below 1.10 mg % ascorbic acid as indicative of hypovitaminosis C, then 43 of the 50 cases of this series must be included in this category. These data serve to corroborate previous findings as to the incidence of hypovitaminosis C in active tuberculosis.[4, 5] None of these 43 cases exhibited a prolongation in decolorization time in comparison with the group in which the blood ascorbic acid was normal. The average decolorization time was 4.7 minutes in the hypovitaminotic cases and 6.3 minutes in the group of 7 cases with normal blood ascorbic acid values. Although the difference in numbers between the 2 groups is rather great, it would appear that no possible correlation between chemical and intradermal tests was possible. Furthermore, the decolorization time of the entire group of 50 cases fell into the normal or saturated periods as stipulated by Rotter and corroborated by Portnoy and Wilkinson, although the blood ascorbic acid was below normal in 43 of the cases. It would seem, therefore, that some other reducing substance or substances other than vitamin C, for example, glutathione, may be responsible for the reduction of the dye in the skin.

Conclusion. No correlation between the fasting blood ascorbic acid and the decolorization time of the dichlorophenolindophenol intradermal test was noted in a series of 50 adult female patients with active tuberculosis, in 43 of whom hypovitaminosis C existed.

10135 P

Reaction of Fulminate with Methemoglobin.

R. D. BARNARD AND W. NEITZEL. (Introduced by A. J. Carlson.)
From the Chemical Laboratory, the Northern Illinois College of Optometry.

The addition of a few drops of saturated aqueous mercuric fulminate or of 2% sodium fulminate solution to about 5 cc of millimolar methemoglobin prepared by the action of ferricyanide on laked human erythrocytes, causes the formation of a scarlet pigment which shows a spectroscopic picture similar to that of cyanmethemoglobin. It differs from cyanmethemoglobin, however, in that the addition of alkali causes a change in the spectroscopic band to those of alkaline methemoglobin, in the case of the fulminate derivative, whereas when cyanmethemoglobin is treated with alkali its band

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[4] Jetter, W. W., and Bumbalo, T. S., *Am. J. Med. Sc.*, 1938, **195**, 362.

[5] Bumbalo, T. S., and Jetter, W. W., *J. Ped.*, 1938, **13**, 334.

persists. The color intensity of the fulminate derivative appears to be about half that of an equimolecular solution of cyanmethemoglobin.

Saturated silver fulminate precipitates methemoglobin from solution, the precipitate being red in color. Where the methemoglobin solution has been prepared by the action of quinhydrone on hemoglobin, mercuric fulminate likewise causes a similar precipitate. This would indicate a difference between the methemoglobins prepared by the action of ferricyanide and that resulting from the action of quinhydrone on hemoglobin. A difference in the behavior of these 2 substances toward hydrogen peroxide has been previously noted.[1]

The fulminates are without effect on the spectroscopic appearance of oxyhemoglobin, carbon monoxide hemoglobin, reduced hemoglobin and of alkaline hematin, whether the last be in alkali carbonate, ammonium hydroxide, triethanolamine or pyridine solution. In the case of the hematin solutions, the fulminates differ from the cyanides in that the latter give rise to cyanhematin.

Solutions of sodium fulminate and of mercuric fulminate which had been oxidized by molecular iodine lost their power of altering the spectroscopic appearance of methemoglobin. Since the fulminate ion and methemoglobin are both essential to the formation of this compound which differs in its reactions from any hitherto described, it is referred to as fulminate methemoglobin.

10136

Growth and Metamorphosis of Anuran Larvae on Thymus Extracts.*

Ralph G. Janes and Albert Segaloff. (Introduced by Warren O. Nelson.)

From the Department of Anatomy, Wayne University College of Medicine, Detroit.

Since the early experiments of Gudernatsch,[1] who produced an acceleration of growth and a delay of metamorphosis of tadpoles fed upon fresh thymus tissue, several investigators have obtained diverse results by feeding thymus glands, either fresh or dried, to amphibian

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[1] Barnard, R. D., Proc. Soc. Exp. Biol. and Med., 1937, **36**, 762.

* Aided by a grant from the Committee on Scientific Research of the American Medical Association. Grant administered by Dr. W. O. Nelson.

[1] Gudernatsch, F., *Arch. f. Entw.-Mech.*, 1912, **35**, 457.

larvæ. Uhlenhuth[2, 3] found that when only thymus tissue was given to newly hatched urodeles, their growth was retarded. This was attributed to the particular food value of the thymus since the animals grew at a normal rate when other food was added to the diet. Contrary to the above observations, Kahn[4] and Romeis[5] found that thymus accelerated the growth of amphibian larvae. Swingle,[6] however, fed fresh and powdered thymus to *Rana* larvae and observed no retardation or acceleration of growth or metamorphosis. The rather extensive literature has been covered in a brief review by Gudernatsch.[7]

Asher and Ratti[8] prepared a thymus extract, thymocrescin, which in their hands has produced marked acceleration of growth as well as a gonadotropic effect upon rats on deficient diets. More recently, Rowntree, *et al.*,[9] have obtained accelerated growth and differentiation in rats through the use of thymus extract (Hanson) in successive generations.

The writers have thought that if a growth-promoting factor is present in such extracts, it may have some effect, beyond that of its food value, upon amphibian larval development.

A clutch of *Rana pipiens* eggs was divided into 3 parts, each of which was placed in one of 3 aquaria containing 2200 cc of water. After hatching, the larvae were fed dried boiled beef liver and boiled lettuce. On alternate days when the water was changed, 10 mg of thymocrescin† was added to one aquarium, 1 cc of Hanson's extract‡ to another, and the third aquarium served as a control. The same number of animals was kept in each of the 3 aquaria at all times, although this number was reduced as necessary to prevent crowding. Linear measurements were made at weekly intervals starting 5/14/38 (Table I) and were continued until 6/18/38, at

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[2] Uhlenhuth, E., PROC. SOC. EXP. BIOL. AND MED., 1917, **15**, 37.

[3] Uhlenhuth, E., *Endocrinol.*, 1919, **3**, 285.

[4] Kahn, R. H., *Arch. f. d. ges. Physiol.* (Bonn), 1916, **163**, 384.

[5] Romeis, B., *Münchener Med. Wochen.*, 1921, **68**, 420.

[6] Swingle, W. W., *Biol. Bull.*, 1917, **33**, 116.

[7] Gudernatsch, F., *Med. Record*, 1937, **146**, 101.

[8] Asher, L., and Ratti, P., *Klin. Woch.*, 1929, **8**, 2051.

[9] Rowntree, L. G., Clark, J. H., Hanson, A. M., and Steinberg, A., *Arch. Inter. Med.*, 1935, **56**, 1.

† The thymocrescin was prepared in this laboratory by the method of Asher.[10] One mg was equivalent to 0.97 g of raw calf thymus. The authors gratefully acknowledge the assistance of Parke, Davis & Co. in performing the preliminary extraction.

[10] Asher, L., *Abderhalden's Handbuch der biologischen Arbeitsmethoden*, 1936, **5**, 929.

‡ Dr. A. M. Hanson has generously supplied us with fresh calf thymus extract every 2 weeks.

TABLE I.
Average measurements in mm of *Rana pipiens* larvæ.

Date:	5/14	5/21	5/28	6/4	6/11	6/18
Control diet	41	48	53	59	63	70
Plus Hanson's extract	41	47	52	58	62	72
” Thymocrescin	39	48	52	58	62	70

which time most of the animals were undergoing metamorphosis.

Animals exhibiting one fore limb were considered certain to complete metamorphosis. The first appearance of such animals was on 6/11/38, at which time one was found in each of the aquaria. Observations were made daily until 6/29/38 (Table II) when the majority of the animals had metamorphosed.

TABLE II.
Daily numbers of *R. pipiens* larvæ noted, newly showing one fore limb.

Date:	6/11	6/15	6/16	6/17	6/18	6/20	6/21	6/22	6/23	6/24	6/25	6/29
Control diet	1	3	2	4	0	2	2	2	2	3	0	0
Plus Hanson's extract	1	4	0	0	1	2	1	3	3	2	1	3
Plus Thymocrescin	1	3	1	5	1	4	0	2	3	2	2	2

From the data presented it appears that under the conditions of the experiment there was no specific effect of either thymus extract upon growth or metamorphosis of *Rana pipiens* larvae. The possibility exists, of course, that some effect might have been obtained if larger quantities of the extracts had been used, or if the material had been administered by injection.

10137

The Jacobson Method for Assay of Liver Extracts.

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In relation to the study of tropical anemias, it was the intention of the authors to follow the method advocated by Jacobson.[1-5] This

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[1] Jacobson, B. M., *Science*, 1934, **80**, 211.

[2] Jacobson, B. M., *J. Clin. Invest.* (Proc.), 1934, **13**, 714.

[3] Jacobson, B. M., *J. Clin. Invest.*, 1935, **14**, 665.

[4] Jacobson, B. M., *J. Clin. Invest.*, 1935, **14**, 679.

[5] Jacobson, B. M., *Brit. J. Exp. Path.*, 1936, **17**, 307.

method, which requires the use of the guinea pig in the bioassay of the antianemic principle present in liver extracts, has been employed also by Miller and Rhoads[6] and Mermod[7] in the study of other antianemic substances. While such investigators as Mermod, Miller and Rhoads, Landsberg and Thompson[8] have followed and accept the reliability of the Jacobson method, Goodman, Geiger and Klumpp[9] failed to corroborate the validity of the method.

A total of 40 normal, adult, male guinea pigs (400-700 g), obtained from several local sources, have been used, from which 20 animals were selected according to the standards recommended by Jacobson. All animals remained healthy during the experiment. The reticulocyte count studied during a 10-day period of standardization at 2- or 3-day intervals did not show figures of over 1.2%, except in 2 animals, in which the reticulocyte count was 2%. The animals were fed a mixture of Michigan State rabbit ration and Para grass before and during the experiment. The least possible amount of blood was obtained each time from the ear vessels. The technic employed by C. Mermod (loc. cit.) was followed and a minimum of 1000 red cells were counted in each determination.

One intramuscular injection of 0.5 cc liver extract (prepared from Lilly's 343 and potent in sprue and of which 1 cc was equivalent to 5 g raw liver) was then given to 10 animals (Group I). Another series of 10 pigs (Group II) received 1 cc liver extract. Reticulocyte counts were made on all animals on the 3rd, 5th, 7th, and 9th day following injections of liver extract.

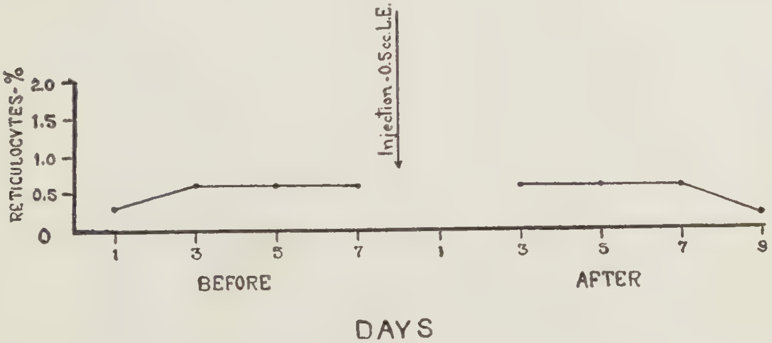

Fig. 1.

Mean reticulocyte percentage obtained in 10 animals (Group I) before and after injection of liver extract.

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[6] Miller, D. K., and Rhoads, C. P., *New England J. Med.*, 1935, **213**, 99.

[7] Mermod, C., *J. Clin. Invest.*, 1936, **15**, 559.

[8] Landsberg, J. W., and Thompson, M. R., *J. Am. Pharm. Assn.*, 1934, **23**, 964.

[9] Goodman, L. S., Geiger, A. J., and Klumpp, T. G., *J. Clin. Invest.*, 1936, **15**, 435.

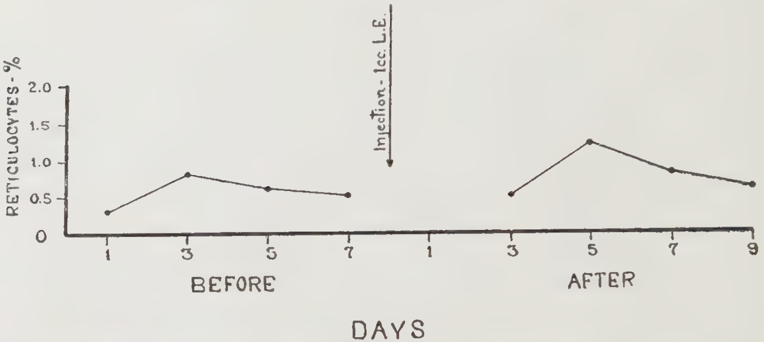

FIG. 2.

Mean reticulocyte percentage obtained in 10 pigs (Group II) before and after injection of liver extract.

Results. The average of reticulocyte counts for all animals obtained on the days following intramuscular injections is given in Figs. 1 and 2.

None of the animals in Group I gave a reticulocyte response of over 2%. A reticulocyte count of 4% was obtained in one of the animals of Group II on the fifth day after injection. This was the only guinea pig of both groups in which the reticulocyte response was significantly greater than 2%, which is considered as the index of reactivity by Jacobson. Two other animals of Group II gave reticulocyte counts of 2.1%, which might be considered as reactive. This rise occurred on the fifth day following injection. In other words, only 3 of the 20 animals used in the experiment were found "reactive" to the method of Jacobson.

The results obtained lead us to consider the method as not sufficiently sensitive in the evaluation of the antianemic principle.

10138 P

Rôle of Inherited Natural Resistance to Tuberculosis.

MAX B. LURIE.

From Henry Phipps Institute, University of Pennsylvania, Philadelphia.

The pioneer studies of Lewis and Wright[1] on inheritance factors in tuberculosis in guinea pigs as well as the writer's observations[2] have prompted a continuance of their studies.

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[1] Wright, S., and Lewis, P. A., *Am. Naturalist,* 1921, **55**, 20.

[2] Lurie, M. B., *J. Exp. Med.,* 1933, **58**, 305.

By brother and sister inbreeding of rabbit groups for 4 to 5 generations families have been developed which exhibit varying, inherited, specific resistance to tuberculosis. This has been determined by exposing the succeeding generations of the different families to artificially infected rabbits in specially constructed large cages separated in the middle by a fine wire mesh screen. On one side of the screen are placed rabbits artificially infected with virulent bovine tubercle bacilli. The bacilli shed by these rabbits, chiefly in their urine, are inhaled by representatives of the different rabbit families which are placed simultaneously on the opposite side of the screen. At stated intervals the contacts are tested with tuberculin. Since by this method the disease acquired by the contacts is nearly always of pulmonary origin, their lungs are X-rayed at the same time. The onset and progress of the disease are thus determined.

It was found, after excluding all known environmental factors that the genetic constitution of the rabbit *per se* may determine the type of disease developed on first natural contact with the tubercle bacillus. Those hereditarily most susceptible develop a disease of

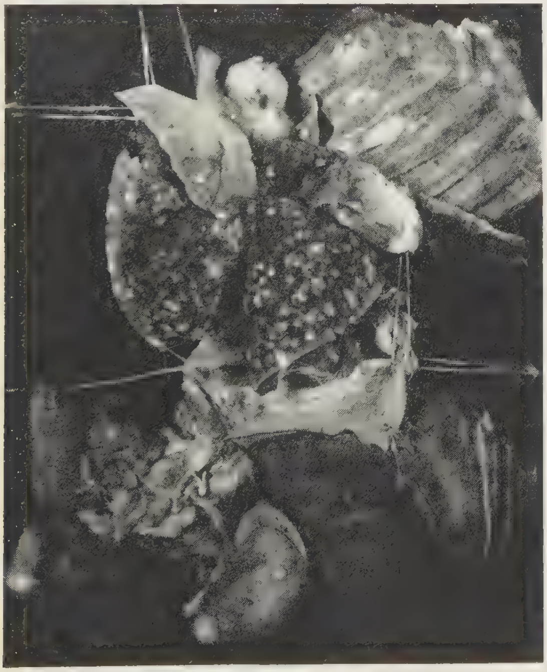

FIG. 1.

Rabbit H2-2 of the H family. Primary focus in upper lobe of right lung; massive caseation of right tracheo-bronchial nodes; miliary tuberculosis of lungs; tuberculosis of ribs, kidney and bone marrow. Duration of disease—3 months.

short duration analogous to infantile tuberculosis. A primary focus develops in the lung, which is accompanied by massive caseation of the draining tracheobronchial lymph nodes. Soon this primary focus undergoes softening and the released bacilli invade the blood stream, so that the animal dies shortly of generalized miliary tuberculosis (Fig. 1). The animals of somewhat greater resistance develop a disease of longer duration analogous to the so-called childhood type with caseous pneumonia, massive involvement of the tracheo-bronchial nodes and large destructive nodular lesions of hematogenous origin in different parts of the body such as the kidneys, eyes, bones, etc. (Fig. 2). The most resistant family, on first exposure to the tubercle bacillus, without previous vaccination, develops a disease of much greater chronicity which is characterized by a localized ulcerative pulmonary phthisis in which the draining tracheo-bronchial lymph nodes and the remaining organs are almost always spared. Anatomically this disease has the distinguishing features characteristic of adult type pulmonary tuberculosis in man

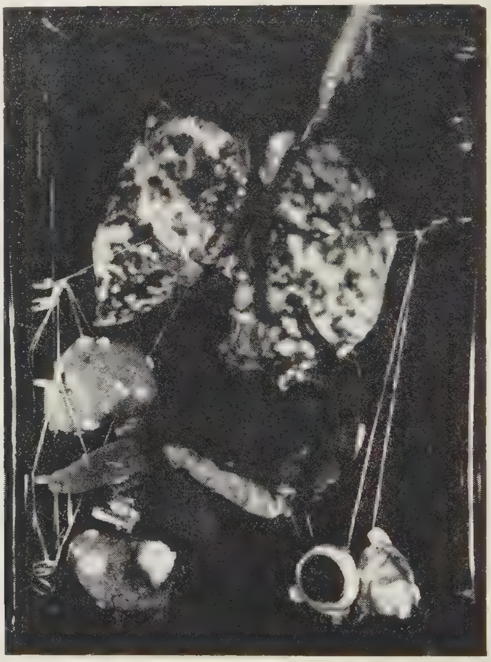

FIG. 2.

Rabbit F2-3 of the F family. Massive caseous pneumonia of both lungs with extensive caseation of left tracheo-bronchial lymph node; extensive tuberculosis of both kidneys; tuberculosis of pleura; destructive tuberculosis of one eye; tuberculosis of trachea and bone marrow. Duration of disease—5 months.

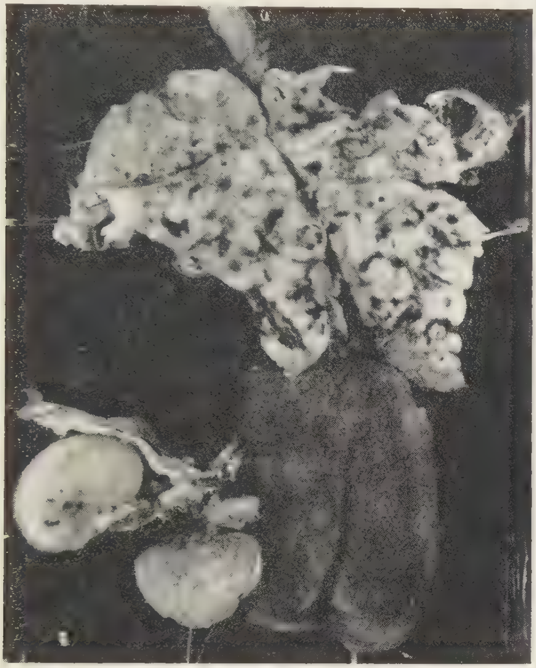

FIG. 3.

Rabbit A2-10 of the A family. Localized ulcerative pulmonary phthisis; draining tracheo-bronchial node tied with string to trachea of small size and grossly normal; healed, small, scarred tubercles in kidneys. Duration of disease—7 months.

(Fig. 3). Hitherto adult pulmonary phthisis has been considered as resulting solely from a reinfection.

Table I summarizes the incidence of caseous tuberculosis of the lymph nodes draining the portal of entry in rabbit families exposed to respiratory contagion. The families are arranged in the order of decreasing resistance. It will be noted that 9 out of 10 rabbits of the most resistant A family have escaped involvement of the hilum lymph nodes entirely, while all of the rabbits of the most susceptible families developed extensive tuberculosis of the draining tracheobronchial lymph nodes. It is noteworthy that a considerable proportion of the rabbits of the B family, of intermediate resistance, developed slight hilum lesions. While the number of observations is small, the difference between the A family and all the others is so great that it suggests an all or none reaction and therefore appears to have considerable significance.

While contagion experiments simulate the mode of infection in man, the dosage of bacilli to which the contacts of the different families are exposed is unknown and inconstant, therefore the resistance

TABLE I.

Incidence of Tuberculous Involvement of Lymph Nodes Draining the Portal of Entry in Rabbit Families Exposed to Natural Respiratory Contagion.

Family	Generation	No. of rabbits	Rabbits with extensive tuberculosis of draining lymph nodes		Rabbits with slight tuberculosis of draining lymph nodes		Rabbits with no gross tuberculosis of draining lymph nodes	
			No.	%	No.	%	No.	%
A	2nd and 3rd	10	0	0	1	10	9	90
B	2nd, 3rd and 4th	7	4	57	3	43	0	0
G	2nd	6	4	67	0	0	2	33
D	2nd and 3rd	5	4	80	0	0	1	20
H	2nd and 3rd	7	6	86	0	0	1	14
F	2nd and 3rd	7	7	100	0	0	0	0
C	2nd and 3rd	7	7	100	0	0	0	0

TABLE II.
Resistance of Rabbit Families to Inoculation of Standard Doses of Bovine Tubercle Bacilli.

.2 mg Ravenel intracultaneously		.001 mg Ravenel per kilo intravenously	
Rabbit No. generation and family	Survival in days	Rabbit No. generation and family	Survival in days
A 3-9	291		
A 3-11	296		
A 3-12	241*		
Avg	276 ± 25		
B 3-5	176	B 3-12	125*
B 3-6	123*	B 3-13	196
B 3-7	196	B 3-16	170
		B 3-15	159
Avg	165 ± 30	Avg	162 ± 25
H 2-19	123	H 2-29	79
H 2-22	108	H 2-31	73
H 2-23	133	H 2-26	78
H 2-24	134	H 2-32	82
Avg	124 ± 21	Avg	78 ± 4

*Tuberculosis complicated by an intercurrent infection.

of these families to tuberculosis has been tested by the inoculation of standard quantities of tubercle bacilli by various routes. Two such titrations of the families A, B, and H are presented in Table II.

It is clear from this table that the A family is most resistant. The B family is of intermediate resistance and the H family of lowest resistance of the 3 families considered. This is the same order of resistance that these families exhibited when they acquired tuberculosis by contagion.

In the succeeding paper further corroborative evidence is presented of the relative resistance of these families to standard doses of tubercle bacilli.

10139 P

Nature of Inherited Natural Resistance to Tuberculosis.

Max B. Lurie.

From the Henry Phipps Institute, University of Pennsylvania, Philadelphia.

In an endeavor to elucidate the physiological bases of the inherited constitutional resistance to tuberculosis in the families discussed in the preceding report the following experiment was performed. About 4 litter mates of each of the third or fourth generations of the families A, B, C, D, F, and H were treated with standard quantities of heat-killed bovine tubercle bacilli. The volume of inflammation that resulted from the primary intracutaneous injection of 1.0 mg of the killed tubercle bacilli served as a measure of the primary inflammatory response of the tissues of the different rabbit families to the injected bacilli. The treatment with dead tubercle bacilli was then continued until a maximum sensitivity developed to a standard quantity of tuberculin injected intracutaneously. The volume of inflammation due to the tuberculin was regarded as a measure of the innate capacity of the tissues to acquire inflammatory responsiveness as a result of sensitization; for under these conditions the antigen responsible for the sensitization was constant, as it could not multiply. After a certain period all these rabbits were then infected uniformly with 0.2 mg of living virulent bovine Ravenel tubercle bacilli intracutaneously. The development of the local lesion and its course were carefully observed, together with the progress of the disease in the draining lymph nodes and internal organs. The volume of inflammation that resulted after intracutaneous injection of a standard quantity of tuberculin at the height of sensitivity during the course of the disease was regarded as a measure of the sensitization of infection or allergy. On the seventy-second day following the virulent infection all the rabbits were given an intracutaneous injection of 0.5 cc of a 1 :5 dilution of India ink. On the next day the area of spread of the ink was determined. This area was regarded as a measure of the permeability of the tissues to particulate matter. The rabbits were then permitted to die from their disease. It may be stated here that preliminary treatment with heat-killed tubercle bacilli did not as a rule materially affect the survival of these animals as determined by previous experience, though it must be admitted that an element of acquired resistance was probably introduced by this treatment. It must be emphasized, however, that sensitization did not alter the relative resistance of these families. The resistance to tuberculosis of each individual rabbit as measured by its survival was correlated with the spread of India ink in its own skin, the primary inflammatory response of its skin to heat-killed tubercle bacilli, the degree of responsiveness of its tissues to sensitization with heat-killed tubercle bacilli, the degree of its sensitization from the virulent infection, the rate of development and healing of its lesion at the portal of entry.

Table I presents the arithmetical average of each of the above observations for each of the 6 families studied. The families are arranged in the order of decreasing resistance. It will be noted in the

TABLE I.

Relation in Rabbit Families between Resistance to Tuberculosis and
(a) Permeability of Skin to India Ink.
(b) Primary Inflammatory Response of Tissues to Tubercle Bacilli.
(c) Acquired Inflammatory Responsiveness of Tissues.
(d) Sensitization of Infection (Allergy).
(e) Rate of Reaction at Portal of Entry.
(f) Rate of Healing at Portal of Entry.

Order of resistance to tuberculosis measured by survival in days after a standard infection	Order of permeability of the skin to India ink. measured by spread of a standard quantity of India ink 24 hr after intracutaneous injection	Order of primary inflammatory response of tissues to tubercle bacilli measured by vol. of inflammation resulting 5 days after primary intracutaneous injection of standard quantity of heat-killed tubercle bacilli	Order of acquired inflammatory responsiveness of tissues to tuberculin measured by vol. of inflammation resulting from injection of a standard quantity of tuberculin after max. sensitization with heat-killed tubercle bacilli	Order of sensitization of infection—allergy measured by vol. of inflammation resulting from injection of standard quantity of tuberculin during height of allergy of infection	Order of rate of reaction at portal of entry measured by vol. of local lesion on fourteenth day after inoculation	Order of rate of healing at portal of entry measured by rapidity with which local lesion attained its max. and % of its healing during course of the disease	
Survival in days	Area in mm²	Volume in mm³	Volume in mm³	Volume in mm³	Volume in mm³	Rapidity in days	Healing in %
A 350	B 153	A 44	A 679	F 1941	B 262	A 28	100
B 164	A 208	B 70	D 634	A 1380	A 615	B 35	76
F 133	F 306	F 84	H 633	D 827	C 645	D 35	74
H 129	D 314	C 88	F 574	H 597	D 661	F 35	70
D 126	H 390	D 90	C 311	B 319	F 733	H 44	68
C 100	C 404	H 107	B 283	C 259	H 749	C 52	68

first column that the A rabbits are outstandingly resistant, the B family is of intermediate resistance, the F, D, and H rabbits are of approximately the same low resistance while the C rabbits are outstandingly susceptible.

The factors with which this resistance is correlated are arranged in increasing or decreasing order depending upon their relationship to resistance. In the second column it is seen that the permeability of the skin to particulate matter tends to be greatest with the most susceptible families and least with the most resistant, though the relationship is not an exact one. It is noteworthy that in normal litter mates of these same rabbit families the order of permeability of the skin to India ink was essentially relatively the same. Thus 3 normal B rabbits spread the India ink in the skin over an average area of 255 mm^2, 6 A rabbits over 355, 6 D rabbits over 379, 3 C rabbits over 575, and 4 F rabbits over an average area of 604 mm^2. It is clear that the presence of tuberculosis reduces the permeability of the skin to India ink as has been noted before[1] but it does not transform a permeable into an impermeable skin. Figures 1 and 2 illustrate the spread of India ink in a susceptible and a resistant rabbit.

In the third column it is seen that, in general, the primary inflammatory response is inversely related to resistance. The greater the primary inflammation produced by the tubercle bacillus in the skin of

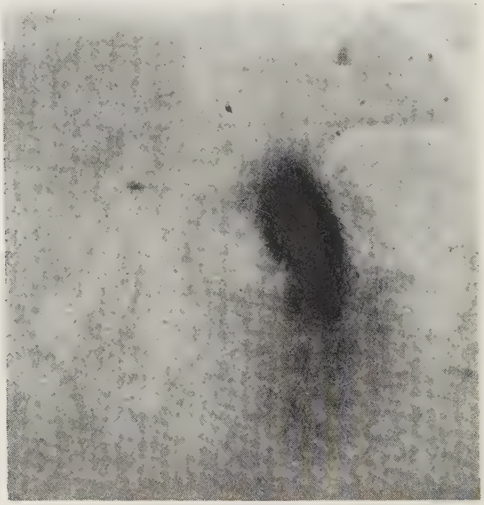

FIG. 1.
The spread of India ink in the skin of rabbit F 3-28 of the susceptible family F.

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[1] Lurie, Max B., *J. Exp. Med.*, 1936, **63**, 923.

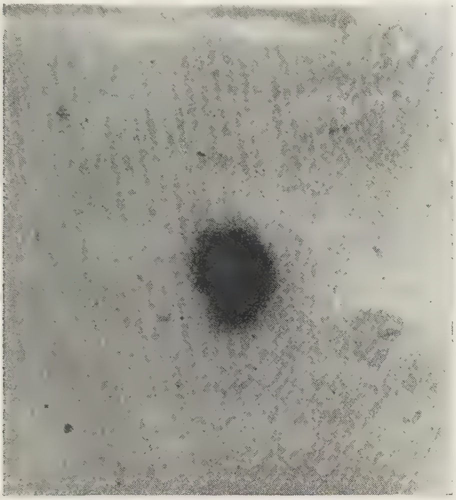

FIG. 2.
The spread of India ink in the skin of rabbit A 4-16 of the resistant family A.

a rabbit, the greater is the susceptibility to tuberculosis. It is clear that this inflammation is to a considerable extent related to the permeability of the skin and, as shown by Thomas and Duran-Reynals,[2] is influenced by the spread of tubercle bacilli in it. The acquired responsiveness of the tissues to tuberculin is not definitely related to resistance. The most resistant family developed the greatest degree of sensitivity to tuberculin on treatment with dead tubercle bacilli, while among the least responsive was found the most susceptible family. The allergy of infection was similarly intense in the most resistant family and least developed in the most susceptible. But in both these factors it is apparent that the relationship is not parallel to resistance as evidenced by families B and D and H in the former, and F and B in the latter relationship. No constant correlation could be found between the permeability of the skin and the degree of inflammatory response to tuberculin on sensitization with dead or living tubercle bacilli. However it must be noted that in 50 unsensitized litter mates of these families the inflammatory response of the skin 48 hours after the injection of concentrated tuberculin is definitely related to the permeability of the skin as shown by Thomas and Duran-Reynals,[2] being most marked in the B family with the least permeable skin and least in the C family with the most permeable skin, while the other families occupy an intermediate position closely related to the order of their respective permeabilities. It is seen in the sixth column that, to a certain extent, the rate of reaction to the inoculation of living tubercle bacilli at the portal of entry is somewhat related to resistance, in the sense that in the susceptible families the local lesion develops more rapidly than in the resistant animals. However, exceptions to this rule are evident in families C and A. It is clear from the last column that the most resistant, the A family, responded at the portal of entry of living tubercle bacilli with a lesion which reached its maximum development in 28 days and healed completely, whereas the most susceptible, the C family, responded with a lesion that attained its maximum development in 52 days and, at death, was far from healed. In families of intermediate resistance the rate of healing at the portal of entry was of intermediate speed. It is to be emphasized that similar though less pronounced differences in the rate of healing at the portal of entry were obtained in these same families which had not undergone preliminary sensitization with heat-killed tubercle bacilli. An analogy has thus been found between naturally highly resistant and artificially immunized rabbits. In both instances the reaction to the tubercle bacillus is abortive as compared with the response on first infection of rabbits of low inherited resistance.

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[2] Thomas, R. M., and Duran-Reynals, F., *J. Exp. Med.*, 1935, **62**, 39.

Figures 3, 4, and 5 illustrate the rate of healing at the portal of entry in 3 rabbits belonging respectively to families of low, intermediate and high resistance to tuberculosis. They depict the skin lesion and drainage lymph nodes on the 60th day after an intracutaneous inoculation of 0.2 mg bovine type tubercle bacilli intracutaneously in 3 rabbits, *viz.*, C 3-16 of the C family which survived 88 days, B 4-22 of the B family, which survived 155 days, and A 4-17 of the A family, which survived 424 days.

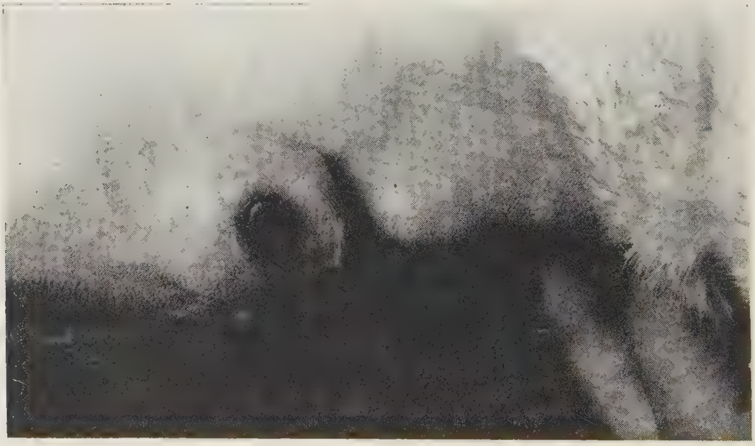

FIG. 3.
Rabbit C 3-16 of the most susceptible C family.

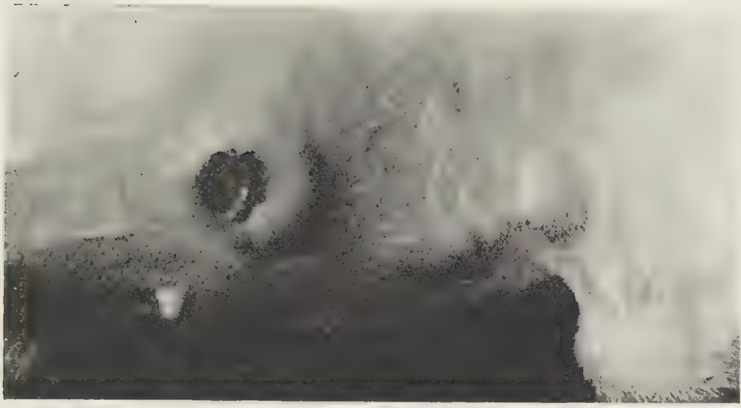

FIG. 4.
Rabbit B 4-22 of the B family of intermediate resistance.

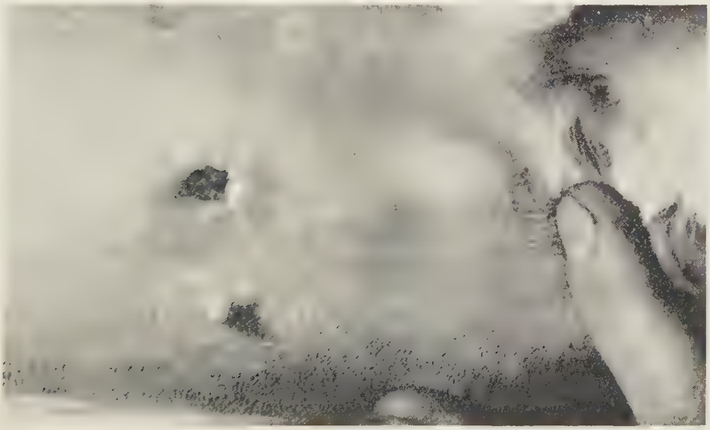

FIG. 5.
Rabbit A 4-17 of the most resistant A family.

The figures in this table help to show that the most resistant families possess more qualities which are associated with resistance, while the most susceptible families have more characteristics associated with susceptibility. It is evident that there is no one factor which by itself determines resistance; rather it is the interplay of these factors which determines resistance. This is particularly evident in the case of family B. While it possesses some of the characters associated with resistance, other factors, as yet not studied, would seem to lower its resistance.

No definite conclusions are drawn at present from these results. They serve as a foundation for further studies.

10140

Skeletal Storage of Fluorine in the Growing Rat Fed Bone Meals of Varying Fluorine Content.*

ROBERT JOHN EVANS AND PAUL H. PHILLIPS. (Introduced by E. B. Hart.)

From the Department of Biochemistry, College of Agriculture, University of Wisconsin, Madison.

In a previous experiment the authors reported that the bones of bovine fetuses and of veal calves were low in fluorine as compared to adult cattle bones.[1] This would seem to offer a means of obtaining a low fluorine bone meal which could be used for certain dietary and therapeutic purposes, especially in view of the fact that commercial bone meals are obtained from bones of cattle from all parts of the country, and may vary therefore in their fluorine content.

To test this suggestion veal calf bones (metatarsal) were obtained from a local packing plant at Madison. The bones were prepared by boiling them to remove the excess trimmings, crushing and extracting with alcohol to remove the fat, and then grinding to a fairly fine powder. This bone meal was analyzed and found to contain 20 parts of fluorine per million. A bone meal was prepared in the same manner from metatarsal bones obtained from University of Wisconsin cattle whose dietary history was known. This bone meal was quite coarse and contained 474 parts of fluorine per million. Two commercial bone meals were obtained. One of these was a special steamed bone meal used for livestock feeding, and the other was supposed to be a higher quality bone meal. They contained 550 and 529 parts of fluorine per million respectively.

Rats were used to test these bone meals, since Ellis and Maynard[2] have shown that the storage of fluorine in the bones of the rat is roughly proportional to the fluorine fed in the ration whether it is present as sodium fluoride or as a constituent of bone meal. The basal ration used had a fluorine content of 4.8 parts per million. It had the following composition:

Yellow corn	76
Crude casein	15
Yeast	3

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* Published with the approval of the Director of the Wisconsin Agricultural Experiment Station. Supported in part by a grant from the Wisconsin Alumni Research Foundation.

[1] Evans, R. J., Phillips, P. H., and Hart, E. B., *J. Dairy Science,* 1938, **21**, 81.

[2] Ellis, G., and Maynard, L. A., PROC. SOC. EXP. BIOL. AND MED., 1936, **35**, 12.

Cod liver oil	1
Iodized salt	1

This basal ration was supplemented with the respective bone meals to be tested on the basis of equal amounts of ash. Five lots of 8 weanling rats each were fed as follows:

Lot I—Basal ration + 5.9 parts of veal calf bone meal.
II—Basal ration + 5.3 parts of a bone meal prepared from bones of University of Wisconsin herd cattle.
III—Basal ration + 4.0 parts of a high quality commercial bone meal.
IV—Basal ration + 4.4 parts of a commercial grade of special steamed bone meal.
V—Basal ration + 2.2 parts of a commercial grade of special steamed bone meal.

The fluorine content of these rations is given in Table I.

TABLE I.
Fluorine Content of Rations Expressed in Parts Per Million.

	Lot I Veal	Lot II U. of W.	Lot III High quality bone meal	Lot IV 4.4% commercial bone meal	Lot V 2.2% commercial bone meal
Fluorine in bone meal	20	474	529	550	550
Fluorine added to the ration by the bone meal	1.2	25.1	21.2	24.2	12.1
Total fluorine in ration	6.0	29.9	26.0	29.0	16.9

The rats weighed from 30 to 50 g each when they were placed on the experimental ration, and were kept thereon for 8 weeks. At the end of this period they were sacrificed, the femurs were removed and analyzed for fluorine. The method of analysis used was a modification of the micro-method of Armstrong.[3] The bones were finely ground in an iron mortar. Duplicate samples of 0.5 g each were weighed out and ashed as previously described.[4] The fluorine was titrated in aqueous solution with 0.02 N thorium nitrate, care being taken to keep the volume of the titrated solution small and its fluorine contents between 5 and 30 micrograms. This method of analysis was found to be very satisfactory for the levels of fluorine encountered in this experiment.

The rats grew well on all of the supplements and showed no gross symptoms of fluorine toxicity or of fluorine deficiency. No noticeable bleaching of the incisors occurred in any group. There was no significant difference in the growth rates of the rats in the different lots.

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[3] Armstrong, W. P., *Ind. and Eng. Chem.*, Anal. Ed., 1936, **8**, 384.

[4] Chang, C. Y., Phillips, P. H., Hart, E. B., and Bohstedt, G., *J. Dairy Science*, 1934, **17**, 695.

The femurs of the rats receiving the veal calf bone meal (Lot I) contained on the average 44 parts of F per million (Table II). Attention is called to the fact that this ration contained *in toto* only 6.0 parts of F per million. On the other hand, Lots II to V were fed rations containing from 3 to 5 times as much fluorine on the basis of total fluorine expressed in parts per million, and yet the skeletal storage as indicated by fluorine in the femur was 7 times greater, or approximately 300 p.p.m. This would seem to indicate that the veal calf bone meal contained less physiologically effective fluorine.

TABLE II.
Fluorine Content of Rat Femurs Expressed in Parts Per Million.

	Lot I Veal calf bone meal	Lot II Bone meal U. of W.	Lot III High quality commercial bone meal	Lot IV 4.4% commercial bone meal	Lot V 2.2% commercial bone meal
	40	306	256	327	285
	38	285	269	352	309
	54	314	313	359	303
	49	280	320	338	293
	46	310	316	350	298
	36	272	252	316	352
	41	265	316	333	305
	50	268	275	313	276
Avg.	44	288	288	336	293

It will be noted from the data in Table I that most of the fluorine in the lot I ration was furnished by the basal ration. This would indicate that much of the fluorine present in veal calf bone meal was apparently combined in such a way that it was not available to the rat for storage in the skeleton.

It was previously indicated that the commercial special steamed bone meal was fed at 2 different levels. It was deemed advisable to feed one bone meal at a lower level, since the 4.4% level is much higher than the bone meal content of practical rations. This was justified as indicated in Table II, for the rats receiving the higher level of bone meal stored very slightly more fluorine in their femurs than did those receiving half this level of bone meal. This would indicate an incomplete absorption or utilization of the bone meal for skeletal growth at the higher level.

The fluorine content of the rations of lot II and lot IV were practically the same. However, the rats of lot IV stored significantly more fluorine than did those of lot II. As was pointed out earlier, the bone meal fed to the lot II rats was quite coarse. Therefore it may have been less readily absorbed by the rats and the fluorine would thus have been less available to the rat. On the other hand, this bone meal contained more organic material and a part of the fluorine may have been bound up in this organic portion and was perhaps less available to the rat for bone storage.

Summary. It was demonstrated again that veal calf bones were low in fluorine. This experiment demonstrated clearly that bone meal prepared from veal calf bones produced femurs with an exceptionally low fluorine content, thus indicating a calcium and phosphorus carrier of high desirability insofar as the fluorine content is concerned. Commercial bone meals were found in this experiment to be responsible for the deposition of considerable amounts of fluorine in the femurs of the growing rat.

These data, further, suggest that the high levels of bone meal feeding in the case of the growing rat resulted in a limited utilization of the minerals as indicated by the storage of fluorine in the femurs and that the fluorine present in bone meal is not selectively absorbed.

Some of these data seemed to indicate that a part of the fluorine in veal calf bones was there in a form not readily available for absorption and storage in the skeleton of the rat.

10141 P

Comparison of Pregnancy Urine Injection and Coitus as Stimuli for Ovulation in the Rabbit.*

B. B. RUBENSTEIN. (Introduced by T. Wingate Todd.)

From the Laboratory of Anatomy and Associated Foundations, Western Reserve University.

Rabbit ovulation, as has been known since 1905,[7] usually follows successful mating by 9-14 hours. The stimulus of coitus is ineffective after transection of the pituitary stalk.[1] Ovulation may also follow electrical stimulation of the central nervous system[10, 6] and of the superior sympathetic ganglia[3] by 18-24 hours. Finally ovulation in the intact animal follows intravenous injection of gonadotropic hormone from either the anterior pituitary or from pregnancy

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* This investigation was supported by a grant from the Rockefeller Foundation.

[7] Heape, W., *Proc. Roy. Soc.*, (London), 1905, B**76**, 260.

[1] Brooks, C. McC., *Am. J. Physiol.*, 1935, **113**, 18.

[10] Marshall, F. H. A., and Verney, E. B., *J. Physiol.*, 1936, **86**, 327.

[6] Harris, G. W., *Proc. Roy. Soc.*, (London), B**122**, 374.

[3] Friedgood, H. B., and Pincus, G., *Endocr.*, 1936, **19**, 710.

urine[18, 4] by 10-24 hours. Hinsey and Markee[9] have pointed out, however, that the gonadotropic hormone injection fails in the absence of the rabbit's own pituitary.

It has been generally assumed that coitus stimulates the output of gonadotropic hormone from the anterior pituitary. The histological changes in the anterior pituitary, described by Wolfe and Cleveland[17] and the bioassays of gonadotropic hormone in the preovulatory-ovulatory period[13, 16] support this theory for the mechanism of ovulation in the intact animal. It has also been assumed that the injection of pregnancy urine or pregnancy urine extracts substitutes, quite simply, for the outflow of pituitary gonadotropic hormone.[5] The logical economy of this theory has proved attractive, although some evidence seems to oppose it.[2, 8]

The evidence gathered up to the present fails to explain why when gonadotropic hormone is injected intravenously it requires a longer time for ovulation to occur than when natural stimuli presumably provoke the discharge of gonadotropic hormone. Among possible explanations only the following is considered in this paper: That the injected material must be metabolized or acts through some intermediary gland.

Rabbits, which had been kept isolated for at least 30 days, were killed by a blow, breaking the neck. The abdomen was opened and both gross and microscopic observation made of the ovaries and, when indicated, of the uterus. The selected tissues were dissected cleanly and excised, part preserved for histological study, the rest placed on ice. Thin slices, rapidly weighed on a torsion balance were placed in Warburg chambers in rabbit serum. The carbon dioxide excreted was absorbed on 0.7% (isotonic) NaOH. The oxygen consumed was measured by the manometric method, over the period when oxygen consumption remained approximately linear (less than 5 hours). The temperature was kept at 37°C carefully controlled.

Ovaries, thyroids, livers and adrenals were preserved in Maximow's fixative, sectioned at 5-7 micra, stained with H and E. Adrenals and pituitaries were fixed in Champy's fluid, and stained with

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[18] Zondek, B., *Klin. Wchnschr.*, 1927, **6**, 248.

[4] Friedman, M. H., *Am. J. Physiol.*, 1929, **90**, 617.

[9] Hinsey, J. C., and Markee, J. E., *Am. J. Physiol.*, 1933, **106**, 48.

[17] Wolfe, J. M., Phelps, D., and Cleveland, R., *Am. J. Anat.*, 1934, **55**, 363.

[13] Smith, P. E., and Engle, E. T., *J. Pediat.*, 1934, **5**, 163; *J. A. M. A.*, 1935, **104**, 553.

[16] Wolfe, J. M., *Am. J. Anat.*, 1931, **48**, 391.

[5] Friedman, M. H., *Endocr.*, 1930, **14**, 328.

[2] Engle, E. T., *J. A. M. A.*, 1929, **93**, 276.

[8] Hill, M. and Parkes, A. S., *Proc. Roy. Soc.* (London), 1930, B**107**, 30.

Severinghaus' triple stain.[11] Sections from all but the last 2 animals have already been examined and found to conform to the descriptions of pituitaries made by Severinghaus.[12]

TABLE I.
Oxygen Consumption of Rabbit Tissues in Cubic Millimeters Per Gram Per Hour.

	During anestrus	During estrus	After coitus			After injection of pregnancy urine			Male
			1-4 hr	5-8 hr	9-12 hr	1-6 hr	7-12 hr	13-18 hr	
Ant. Pit.	470	600	500	490	440	560	1870	770	420
Adrenal	300	370	350	530	510	730	750	1610	300
Liver	460	420	340	300	510	540	370	560	350
Ovary	320	390	400	440	460	350	510	570	
Thyroid	500	550	450	550	350	550	650	380	400

Table I presents a summary of the data. Each value is an average of from 3-6 experiments. The range of values was comparatively narrow, not exceeding ±25% of the average, which is reasonable since there was inevitably some variation in thickness of slice and in the amount of relatively inactive connective tissue in the samples studied.

Of all the tissues studied, only the anterior pituitary and adrenal show very large changes. The oxygen consumption of the anterior pituitary is low in anestrus, increases markedly in estrus, and falls off to about its anestrus levels in the preovulatory period. The values reported are about 20% lower at each level than those found in the rat by Victor and Anderson.[14] A few experiments confirmed the reported values on rats. After pregnancy urine injections, however, the oxygen consumption of the anterior pituitary rises to about 3 times its estrous level. An increase was foreshadowed in the cytological changes reported by Severinghaus[12] and in the reports of Victor and Anderson.[15] A change in oxygen consumption so large that it cannot be attributed entirely to the estrogen present in 5 cc of pregnancy urine is significant. It must indicate an extraordinary increase in the activity of the anterior pituitary. Probably enough hormone is produced and excreted to result in ovulation without the necessity of attributing even part of the ovulating dose to the gonadotropic hormone present in the pregnancy urine.

What the rôle of the adrenal may be I cannot guess at this time. The behavior of the other tissues, however, which corresponds to that normally found in the estrus-ovulatory period, leads me to believe that after activation of the rabbit's own pituitary, the mechanism of ovulation is probably the same as after coitus.

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[11] Severinghaus, A. E., *Anat. Rec.*, 1932, **53**, 1.

[12] Severinghaus, A. E., *Proc. Assn. Res. Nerv. and Ment. Dis.*, 1936, **17**, 69.

[14] Victor, J., and Andersen, D. H., *Am. J. Physiol.*, 1936, **115**, 130.

[15] Victor, J., and Andersen, D. H., *Am. J. Physiol.*, 1937, **120**, 154.

Summary. The response to pregnancy urine of the rabbit anterior pituitary is one of increased oxygen consumption in the 7-12-hour interval after injection. The inference may be drawn that the effect of pregnancy urine is to activate the anterior pituitary leading to the output of an effective dose of rabbit hormone after an interval of 12-24 hours.

10142

The Stage of Mitosis at which Chromosomes are Rendered Less Sensitive to X-rays by Ammonia.

Alfred Marshak. (Introduced by Shields Warren.)

From the Laboratory of Pathology, the New England Deaconess Hospital, Boston.

On the basis of data obtained in previous experiments, it has been postulated that the marked sensitivity to X-rays of chromosomes at the onset of the prophase may be attributed to the neutralization by negative electrons of positive charges carried by the adjacent surfaces formed upon division of the chromonemata.[1] Later experiments showed that in keeping with this hypothesis, treatment with CO_2 did not alter the reaction of chromosomes to X-rays, while NH_4OH greatly reduced the number of chromosome abnormalities produced by a given dose of X-rays.[2] The theory further required that in normal mitosis the positive charges appear at the onset of the prophase and then disappear during the course of the prophase, presumably as a result of the combination of the positively charged groups with nucleic acids. In accordance with this hypothesis a penetrating base such as NH_4OH would be expected to reduce the X-ray sensitivity of chromosomes primarily at the onset of the prophase. The experiments to be described were designed to determine whether these expectations would be fulfilled.

Onion seedlings* were irradiated with X-rays and at various intervals after irradiation the root tips examined for the percent anaphases which had no chromosome abnormalities. Similar seedlings were immersed in 0.0025 N NH_4OH for 30 minutes and then irradiated. The technic used has already been described.[2] Both groups of seedlings were given 80 roentgens of X-rays. The source of radiation was an oil-cooled X-ray tube† with a tungsten target which was operated at 200 K.V. and 25 ma. with 2 valve rectification. The filtration by the glass, oil and bakelite was equivalent to 2 mm Al. and 0.25 mm Cu. The effective wave-length was 0.235Å. The distance from target to seedlings was 80 cm and the time for irradiation 112 seconds. By accident one lot of seedlings received only 75 r

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[1] Marshak, A., *Proc. Nat. Acad. Sci.*, 1937, **23**, 362.

[2] Marshak, A., Proc. Soc. Exp. Biol. and Med., 1938, **38**, 705.

* Sweet Spanish, Riverside Strain, Ferry-Morse Seed Company.

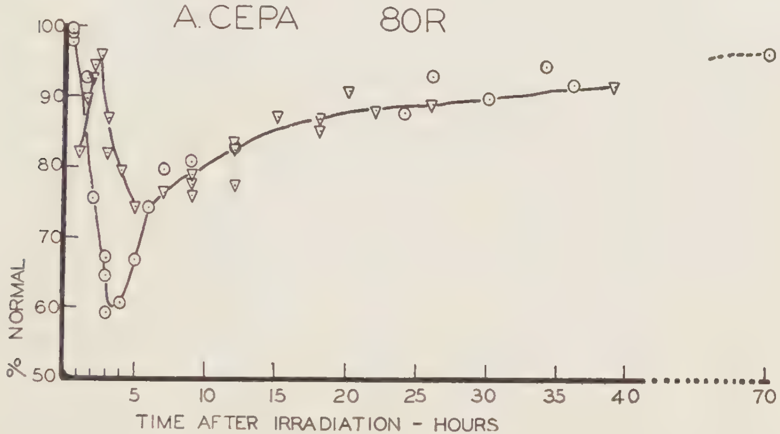

FIG. 1.

Ordinates, % normal anaphases. Abscissæ, time in hours after irradiation. Circles represent data from seedlings treated only with X-rays, the triangles seedlings treated with ammonia and X-rays. With the exception of 3 points all were obtained from seedlings given 80 roentgens. The 3 exceptions are the topmost circle at 3 hours and the circles at 7 and 9 hours which by accident received only 75 roentgens.

The results obtained are shown in Fig. 1, where the percent normal anaphases are plotted as a function of time after irradiation. The data upon which these curves are based are shown in Tables I and II. Counts of 700 anaphases in untreated control seedlings showed 0.7% abnormal with respect to chromosome separation. As has been previously described for this and other species, there is a pronounced minimum in the percent normal anaphases at 3 hours for cells treated only with X-rays. In marked contrast, the seedlings treated with ammonia and X-rays showed a sharp maximum in the percent normal anaphases at 2.5 hours after irradiation or 3 hours

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† Purchased from the Picker X-ray Corporation. Style 1935, Serial No. 109, tube No. 10264.

TABLE I.

X-ray—Time Curve—Experiments No. 64 = 80 r; 171 = 80 r; 177 = 75 r.

A. cepa.

Time after X-ray in hr	Exp. No.	Slide No.	n	a	Total	% n
0.5	64	517	350	9	359	97.5
	171	1555	565	4	569	99.5
	64	526	396	3	399	99.4
1.0						
1.5	64	527	266	22	288	92.4
2.0	171	1556	163	52	215	75.8
3.0	177	1577	248	122	370	67.0
	171	1557	222	154	376	59.1
	64	528	148	81	229	64.5
4.0	171	1558	206	135	341	60.4
5.0	171	1559	238	118	356	66.9
6.0	64	529	159	56	215	74.2
7.0	177	1578	445	113	558	79.9
9.0	177	1579	345	81	426	81.0
12.0	177	1580	601	125	726	82.9
24.0	64	530	278	37	315	88.3
26.0	171	1560	610	42	652	93.5
30.0	64	532	360	39	399	90.3
34.0	64	535	757	40	797	95.0
36.0	64	523	649	54	703	92.4
70.0	64	536	701	20	721	97.2

after the beginning of the ammonia treatment. By 5-6 hours the curves for the two groups are identical.‡

It has been shown previously that the cells from which the minimum percent abnormal anaphases are derived at 3 hours after irradiation were in the onset of the prophase during the exposure to the X-rays. Accordingly the cells seen in anaphase at 5 to about 25 hours after irradiation were in various phases of development in the resting stage when exposed. One may conclude, therefore, that ammonia does not alter the sensitivity to X-rays of chromosomes in the resting stage.

The points at 1-2 hours after irradiation represent counts of cells

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‡ The points obtained from the lot of seedlings which received only 75 r are represented in Fig. 1 by the topmost circle at 3 hours and the circles at 7 and 9 hours. As was to be expected they were somewhat higher than the curve for 80 r but are considered sufficiently close to be included in this group.

TABLE II.
NH_4OH + X-ray—Experiments Nos. 164, 165, 176, 178, 179, 181.
A. cepa.

Time after X-ray in hr	Exp. No.	Slide No.	n	a	Total	% n
0.5						
1.0	164	1526	481	108	589	82.0
1.5	178	1583	462	52	514	89.9
2.0	178	1584	168	14	182	92.4
	164	1529	341	24	365	94.5
2.5	178	1585	71	3	74	96.0
3.0	176	1569	153	34	187	82.0
3.0	164	1527	387	58	445	87.0
4.0	164	1530	301	78	379	79.5
5.0	176	1570	208	72	280	74.4
7.0	176	1571	279	87	366	76.3
9.0	179	1595	114	33	147	77.6
	176	1572	237	75	312	76.0
	181	1603	75	20	95	79.0
12.0	178	1586	454	89	543	83.6
	176	1573	439	126	565	77.6
	178	1587	615	126	741	83.0
15.0	178	1588	823	118	941	87.4
18.0	178	1589	744	110	854	87.1
	178	1590	434	73	507	85.5
20.0	178	1591	718	68	786	91.3
22.0	176	1574	316	41	357	88.6
26.0	176	1575	829	98	927	89.5
39.0	178	1592	726	63	789	92.1

which were in the prophase when irradiated. The percent normal anaphases in the cells receiving ammonia and X-rays at 1 and 2 hours after irradiation are approximately the same as for cells treated only with ammonia (Table IV in [2]). For the seedlings receiving ammonia and X-rays the values are 82.0 and 93.0% respectively and for those receiving only ammonia they are 87.0 and 92.4, These results show that ammonia completely protects the chromosomes from the action of the X-rays during the earlier prophases, and in accordance with the hypothesis indicate the presence of some positive charges in the chromonemata during these stages. In Fig. 1 it can be seen that at 1.5 hours after irradiation there is little difference between cells treated with X-rays alone and those receiving ammonia and X-rays. By 2 hours after irradiation there is a wide divergence between the values of the 2 groups indicating that in the normal mitosis the positive charges disappear quite rapidly during the early prophase.

Irradiation with X-rays not only produces chromosome abnormalities, but reduces the number of cells entering the prophase. The data obtained show a marked reduction in the number of cells in anaphase with time, which reaches a minimum at about 3 hours after irradiation.[1] If the ammonia is considered to act on the nuclei within a few minutes after the seedlings are immersed, the maximum in the percent normal anaphases comes at 3 hours after the action of the ammonia. Ammonia also reduces the number of cells in anaphase from about 1,100 per 30 roots at 0.5 hour after immersion to a minimum of 370 at 3 hours after irradiation (Table IV). This is taken to indicate that ammonia not only suppresses the positive charges on the dividing chromonemata, but also inhibits the onset of the prophase. However, in sharp contradistinction to X-rays ammonia produces the most chromosome abnormalities in the metaphase when the X-ray action is so slight as to be hardly or not at all detectible.

These results, therefore, support the hypothesis that positively charged surfaces are developed in the chromonemata at the onset of the prophase and are gradually neutralized during the prophase. They also suggest that ammonia, like X-rays, inhibits cells from entering the prophase.

10143

Changes in Protein-Content of Bacterial Suspensions During Lysis and Autolysis.

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The lysis of bacteria by phage has been considered as a simple plasmolysis, quite different from autolysis, and in fact many attempts to detect protein split products in the solution after lysis have failed. Bronfenbrenner and Hetler,[1] however, did find evidence for the appearance of protein split-products in solution after lysis and hence concluded that lysis was due to hydrolysis of the protein of the bacterial cell.

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[1] Bronfenbrenner, J., and Hetler, D. M., *J. Exp. Med.*, 1928, **48**, 269.

Meyer, Palmer, Thompson, and Khorazo[2] have reported the appearance of non-protein nitrogen during lysis of Sarcina by lysozyme.

The detection of minute amounts of protein split-products in culture-media is a difficult matter but the loss of protein may be readily and accurately followed provided the culture-medium itself contains little or no protein.

In the present experiments bacteria were grown on the protein-free yeast-extract media previously described.[3] The quantity of cells in suspension was determined by comparing the turbidity of the suspension in water with that of a standard casein-suspension by means of a Klett photoelectric colorimeter. This figure was called "water-insoluble protein" and was found to agree quite closely with the protein-content of growing bacterial suspensions as determined by Kjeldahl determinations of the centrifuged and washed sediment.

A second turbidity-measurement was made on a sample of the suspension diluted in 2% trichloracetic acid. This figure is called "total protein."

The relative changes in those 2 quantities is best shown by plotting the water-insoluble protein of each sample against the total protein for the same sample. The changes in water-insoluble protein and

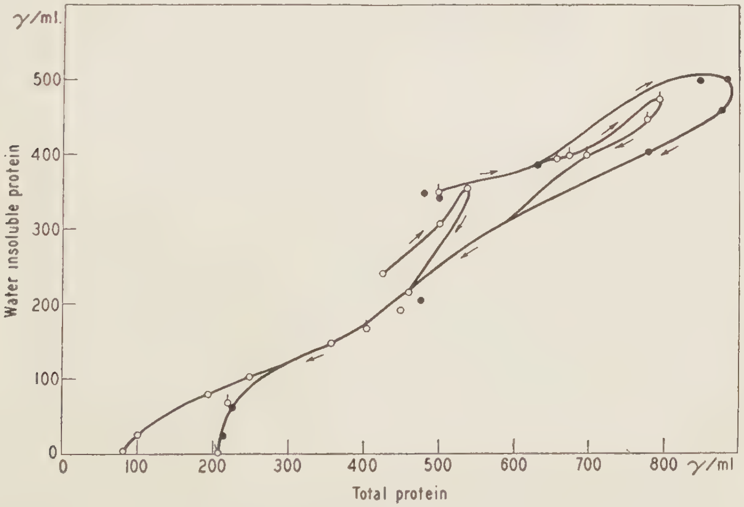

FIG. 1.

Changes in water-insoluble and total protein during lysis of *Staphylococcus musca* by bacteriophage. The different symbols in graphs denote different experiments.

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[2] Meyer, K., Palmer, J. W., Thompson, R., and Khorazo, D., *J. Biol. Chem.*, 1936, **113**, 479.

[3] Northrop, J. H., *J. Gen. Physiol.*, 1938, **21**, 335.

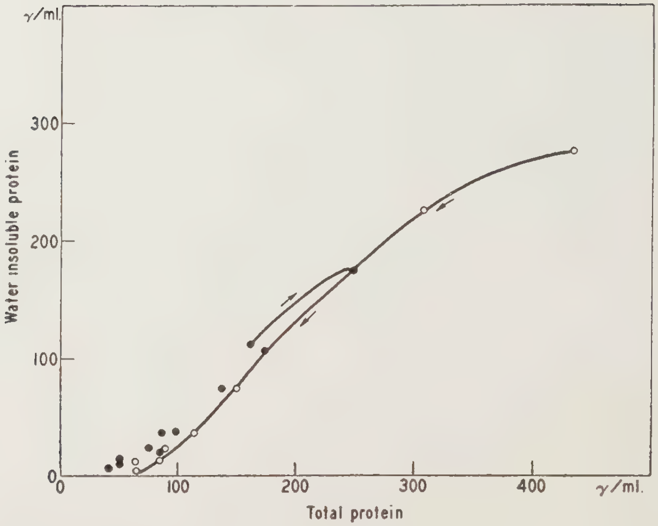

FIG. 2.

Changes in water-insoluble and total protein during lysis of *Staphylococcus aureus* by bacteriophage.

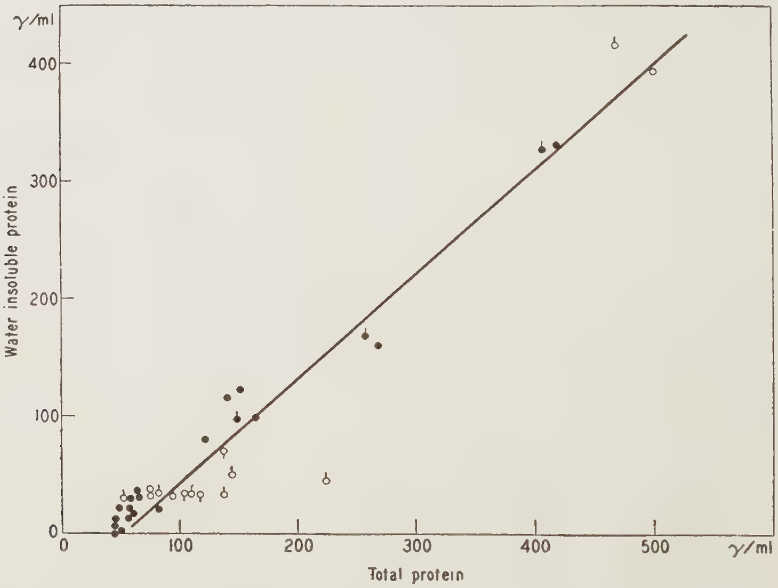

FIG. 3.

Changes in water-insoluble and total protein during autolysis of *Staphylococcus aureus* under anaërobic conditions.

total protein during growth and lysis have been determined for *Staphylococcus aureus, Staphylococcus musca, B. coli,* and *B. megatherium.* The changes in the staphylococcal cultures during autolysis

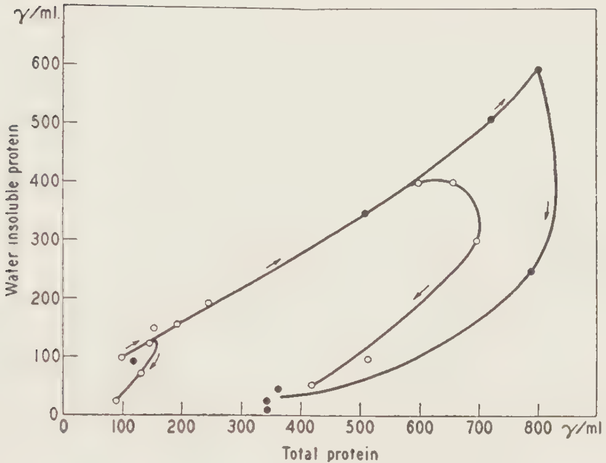

FIG. 4.

Changes in water-insoluble and total protein during lysis of *B. coli* by bacteriophage.

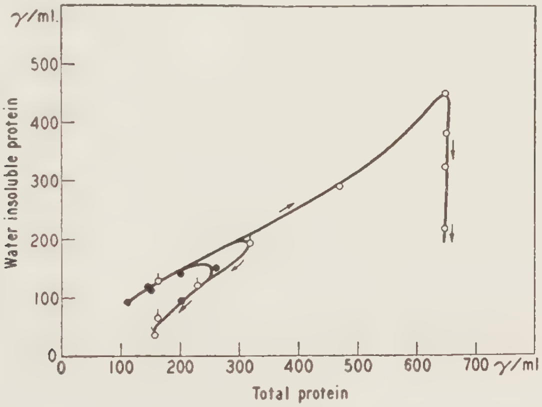

FIG. 5.

Changes in water-insoluble and total protein during lysis of *B. megatherium* by bacteriophage.

under anaërobic conditions[4] have also been determined. The results are shown in Figs. 1-5. The direction of the change in concentration is indicated by the arrows.

The results show that in the case of the staphylococcal suspensions the figures for total protein and water-insoluble protein are nearly equal during either growth, lysis, or autolysis (Figs. 1, 2, 3). No protein is liberated, therefore, when the cells break down. A very small amount of protein remains in solution after the aqueous solution is clear but there is no significant difference between the curves for autolysis and for lysis. The protein must, therefore, be hydrolyzed immediately upon disintegration of the cell.

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[4] Jaumain, D., *Compt. rend. Soc. Biol.*, 1922, **87**, 790.

In the case of *B. coli* (Fig. 4) or *B. megatherium* (Fig. 5), however, some protein does appear in solution as the cells disintegrate, since the decrease in water-insoluble protein during lysis is faster than the decrease in total protein. The total protein also decreases before lysis is complete and at the end of the process the solution is practically protein-free.

The coli and megatherium could not be made to autolyze.

According to these results there is no general relationship between lysis and protein-hydroysis with different basterial suspensions. No protein appears in solution during lysis or autolysis of staphylococcus while some does appear during lysis of *B. coli* or *B. megatherium.*

The writer is indebted to Dr. A. P. Krueger for the *Staphylococcus aureus* cultures and phage and to Professor André Gratia for the *B. coli* and *B. megatherium* cultures and their accompanying phages.

10144 P

Immediate Effects of Coronary Sinus Ligation on Dynamics of Coronary Circulation.

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It has been suggested by Gross,[1] Robertson,[2] and Ungerleider[3] that ligation of the coronary sinus or coronary veins might materially aid hearts whose normal blood supply had been partially removed. Fortunately, such a concept can be tested physiologically and hence an experimental study was made of the possible practical value of such a procedure.

In order to obtain a better understanding of what happens dynamically in the coronary vascular bed following ligation of the coronary venous system, the following have been studied in 28 acute experiments on dogs and largely by optical methods: (1) the coronary inflow, (2) the peripheral coronary pressure, (3) intravenous pressure, (4) the retrograde blood flow in the coronary arteries, together with oxygen determinations, (5) the ability of the myocardium to maintain contractions after its normal blood supply has been removed. The results which have been obtained follow.

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[1] Gross, L., Blum, L., and Silverman, *J. Exp. Med.*, 1937, **65**, 91.

[2] Robertson, H. F., *Am. Heart J.*, 1935, **10**, 533.

[3] Ungerleider, H., Kerkhof, A., and Fahr, G., PROC. SOC. EXP. BIOL. AND MED., 1938, **37**, 703.

After coronary sinus ligation the reduction in coronary inflow (by the method of differential pressure curves) is considerable in the left coronary, but not in the right.

The contour and time relations of the peripheral coronary pressure are similar to those obtained under normal conditions[4] but both the diastolic and systolic resistances are greatly elevated in the left coronary. The latter value approaches, equals or exceeds the aortic systolic pressure simultaneously recorded. This pressure increment has its source largely in the other non-occluded coronaries.

The intravenous pressure (in a coronary vein or in the coronary sinus) rises from control values of say 10/2 mm Hg. to figures which during systole approach or exceed the aortic systolic and whose diastolic value reaches 20 to 40 mm Hg. This pulse is patterned in time and contour after the peripheral coronary pressure while its ordinate values are generally slightly greater.

The retrograde blood flow is markedly elevated in the ramus descendens anterior from a maximum normal value approximating 1 cc per minute to values as high as 39 cc per minute. The maximum peripheral flow is reached in from 10 to 30 minutes and following sinus release does not immediately return to control backflow figures. This increase does not occur in the right coronary. The blood is highly unsaturated, containing only 3-4 volumes percent oxygen, while the coronary sinus approximates 8 volumes percent oxygen.

Such a volume of blood with its low oxygen content is not sufficient for a region of myocardium whose coronary has been occluded, for myographic records show systolic extension within 2-3 minutes after occlusion of a major left coronary ramus, while the peripheral coronary is either closed or allowed to bleed.

In view of these findings it is believed that after sinus blockage an additional mean volume of blood coming largely from the coronary arteries is trapped in the venous system, thus raising the pressure in both venous and arterial vascular beds. However, there are potent drainage channels still remaining and presumably largely venous, since the left coronary inflow is only moderately reduced and the myocardium fails to contract following left coronary artery ligation. Consequently, as a result of the combined occlusion of the sinus and a left coronary ramus, dynamic conditions are created, such that there should be a sizable blood flow from non-occluded coronaries through their capillary beds into the large veins and then by Thebesian and arterioluminal vessels into the heart cavities.

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[4] Gregg, D. E., Green, H. D., and Wiggers, C. J., *Am. J. Physiol.*, 1935, **112**, 362.

If the peripheral coronary artery is closed (not allowed to bleed) it is believed that no adequate anatomical circuit exists to route this venous blood through the capillary bed of the occluded coronary, whereas if the peripheral coronary is opened a portion of this blood is routed in retrograde fashion from the venous system through the capillary bed of the occluded artery. Since ligation of the cardiac veins does reduce considerably the normal left coronary inflow and does not prevent failure of contraction in a myocardial area whose normal blood supply has been acutely removed, such a procedure cannot be regarded as a method of choice for encouraging the blood supply to a potentially infarcted area.

10145

Inhibitory Action of Narcotics on the Histamine Contraction of Plain Musculature.

LAURENCE FARMER. (Introduced by A. Bernhard.)
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As was first shown by Besredka[1] narcosis may prevent fatal anaphylactic shock in guinea pigs. This effect of the narcotic is not due to its action on the central nervous system as presupposed by Besredka, but is probably attributable to its peripheral action on the bronchial musculature (Farmer[2]). In *in vitro* experiments I[3] was able to demonstrate that narcotics (urethane, chloralose) inhibit anaphylactic contraction of sensitized uterine strips of guinea pigs without inhibiting desensitization of the muscle.

Dale's[4] theory of anaphylaxis assumes that anaphylactic contraction of plain musculature is caused by liberated histamine or "histamine-like" substances. In my experiments (l. c.) anaphylactic contraction of the uterine strips was prevented through the inhibitory action of the narcotics on the histamine contraction of plain musculature (Saito,[5] Katz[6]).

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[1] Besredka, *Ann. de l'Inst. Pasteur*, 1907, **21**, 957.

[2] Farmer, *J. Immunol.*, 1937, **32**, 195.

[3] Farmer, *J. Immunol.*, 1937, **33**, 9.

[4] Dale, *Lancet*, 1929, I, 1179, 1233, 1285.

Several authors[7] have shown that narcotics cause loss of tonus of excised strips of plain musculature (bronchi, intestine, uterus, ureters). This loss of tonus probably explains why histamine fails to cause contraction of plain musculature in the presence of narcotics. The following experiments were undertaken: (1) to compare the inhibitory action of narcotics belonging to various chemical groups on the histamine contraction of plain musculature; and (2) to ascertain the effect of substitution of radicals within these groups.

Method. Young female guinea pigs, weighing between 225 and 315 g, were used as test animals. They were fed a diet consisting of carrots, lettuce and oats. The animals received their last feeding 18 to 24 hours prior to the experiment and were killed by a blow on the head and bled by severing the carotids. Immediately after killing the animal, a piece of ileum about 15 cm long was excised and kept in an oxygenated Ringer's solution of 38° to 39°C. The experiment was carried out by suspending vertically a strip of approximately 2.5 cm length in an oxygenated bath solution of 38° to 39°C. The suspended strip was attached to a lever with a writing point and the muscular actions were recorded on a slowly revolving kymograph. The bath solution was either 50 cc Ringer's solution or 50 cc narcotic solution. The Ringer's solution was made up as follows: NaCl 9.0 g, KCl 0.40 g, $CaCl_2$ 0.24 g, $MgCl_2$ 0.005 g, $NaHCO_3$ 0.5 g, dextrose 1.0 g, distilled water ad 1000 cc. The dextrose and $NaHCO_3$ were added shortly before using the solution. All chemicals were of "tested purity." Water was redistilled in a glass still. The narcotic solution was prepared by dissolving the narcotic in Ringer's solution.

Control experiments. The controls were carried out by suspending an intestinal strip in 50 cc Ringer's solution. After 8 to 10 minutes 0.5 cc of a 1×10^{-6} histamine phosphate (Parke, Davis) solution was added to the bath. In this concentration (1×10^{-8}) histamine causes very marked contraction of the strip.

Narcotic experiments. Eight to 10 minutes after suspension of the intestinal strip in the narcotic solution (50 cc) 0.5 cc of a 1×10^{-6} histamine phosphate solution was added. If contraction took place, a new strip was suspended in a stronger narcotic solution and increasing amounts of histamine were added to the bath. The procedure was repeated, fresh strips and fresh narcotic solutions being used, until a narcotic concentration was elicited which completely inhibited contraction of an intestinal strip after addition of $315\,\gamma$ histamine phosphate (end concentration of the histamine 1.58×10^{-5}). The concentration of this narcotic solution is designated as the "inhibiting concentration" in the tables. It was felt that addition of an amount of histamine so greatly in excess of that used in the control experiment tended to eliminate variations in the response of individual strips.

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[5] Saito, *Z. f. ges. exp. Med.*, 1924, **41**, 570.

[6] Katz, *Arch. f. exp. Path.*, 1929, **141**, 366.

[7] Trendelenburg, *Arch. f. exp. Path.*, 1912, **69**, 79; Redonnet, *Arch. Internat. d. Pharmacody e. d. Therap.*, 1925, **30**, 322; Gruber, *J. Pharm. and Exp. Ther.*, 1926-27, **30**, 149; Fromherz, *Arch. f. exp. Path. u. Pharm.*, 1933, **173**, 78; Frey, *Arch. f. exp. Path. u. Pharm.*, 1931, **159**, 163; Mercier, Krijanovsky and Paganelli, *C. R. soc. biol.*, 1937, **121**, 463.

Results. Twenty-one narcotics, belonging to 6 different chemical groups, were examined. Seventy-two guinea pigs were used and 196 individual experiments were carried out. The results of these experiments, which were very uniform, are summarized in Tables I-IV. Furthermore, the inhibiting effect of ephedrine sulphate on the histamine contraction of intestinal musculature was determined in the above described manner. In a concentration of 1:400 ephedrine sulphate completely inhibits contraction of an intestinal strip after addition of $315\,\gamma$ histamine. The inhibiting effect of this ephedrine sulphate concentration was designated as 1 and the inhibiting effects of the various narcotic solutions were compared with it.

TABLE I.
Inhibiting Action of Barbiturates on the Histamine Contraction of Intestinal Musculature.

Substance	Inhibiting concentration	Inhibiting effect compared with Ephed. Sulph. = 1
Diethyl barbituric acid (Veronal)	1:400	1.0
*Ethylisopropyl ’’ ’’ (Ipral ‘‘acid’’)	1:500	1.25
*N-Butylethyl ’’ ’’ (Neonal ‘‘acid’’)	1:2000	5.0
Isoamylethyl ’’ ’’ (Amytal)	1:4000	10.0
Phenylethyl ’’ ’’ (Luminal)	1:1000	2.5
Na-Diethyl ’’ ’’ (Sodium-Veronal)	1:80	0.2
Na-Isoamylethyl ’’ ’’ (Sodium-Amytal)	1:2000	5.0
Na-Phenylethyl ’’ ’’ (Sodium-Luminal)	1:80	0.2
Diallyl ’’ ’’ (Dial)	1:500	1.25
*Allylisopropyl ’’ ’’ (Alurate)	1:1000	2.5
*Isobutylallyl ’’ ’’ (Sandoptal)	1:2000	5.0

*The author wishes to express his thanks to the following firms which kindly furnished some of the drugs used: Abbott Laboratories (Neonal ‘‘acid’’); E. R. Squibb & Sons (Ipral ‘‘acid’’); Sandoz Chemical Works, Inc. (Sandoptal); F. Hoffmann-La Roche & Co. (Alurate).

TABLE II.
Inhibiting Action of Urethanes on the Histamine Contraction of Intestinal Musculature.

Substance	Inhibiting concentration	Inhibiting effect compared with Ephed. Sulph. = 1
Ethylurethane	1:66	0.165
n-propylurethane	1:133	0.33
n-butylurethane	1:400	1.0
Phenylethylurethane	1:2000	5.0
Trichlorourethane	1:1000	2.5

TABLE III.
Inhibiting Action of Sulfones on the Histamine Contraction of Intestinal Musculature.

Substance	Inhibiting concentration	Inhibiting effect compared with Ephed. Sulph. = 1
Sulfonal	1:200	0.5
Trional	1:400	1.0

TABLE IV.
Inhibiting Action of Chloretone, Paraldehyde, Morphine, on the Histamine Contraction of Intestinal Musculature.

Substance	Inhibiting concentration	Inhibiting effect compared with Ephed. Sulph. = 1
Trichloroisobutyl alcohol (Chloretone)	1:1000	2.5
Paraldehyde	1:100	0.25
Morphine hydrochloride	1:400	1.0

As shown by these tables, all of the examined narcotics exercise a marked inhibiting action on the histamine contraction of intestinal musculature. The suspended intestinal strips showed very pronounced relaxation, which was not overcome by addition of histamine. It is interesting to note in this connection that morphine hydrochloride showed the same inhibiting action on histamine contraction, although it does not prevent fatal anaphylactic shock in guinea pigs. As demonstrated by Trendelenburg (l. c.) it has a marked relaxing effect on excised bovine bronchial musculature.

Substitution of aliphatic radicals with increasing C-atoms enhances the relaxing and inhibiting action of narcotics. Substitution of a phenyl group or halogenation have the same effect (see Tables I, II, III).

In Table V the pH of a number of inhibiting narcotic solutions are registered. As seen from this table the pH of the narcotic solution is not the cause of its inhibiting action. Although the concentration of an inhibiting ethylurethane solution is approximately 30 times as great as the concentration of an inhibiting phenylurethane solution their respective pH are 8.6 and 8.7. Furthermore, the pH of an inhibiting ethylurethane solution is the same as that of the Ringer's solution (8.6). However, the hydrogen ion concentration of a narcotic solution can exert a certain effect on its inhibiting action. As shown in Table I sodium veronal, sodium amytal, and sodium luminal which are all decidedly alkaline (Table V) have a smaller inhibiting effect than the corresponding acids. This is in accordance with the observations of Gruber (l. c.) who found that increasing the pH of the bathing fluid heightened the tonus of the tissues, whereas a decrease in the pH brought a fall in tonus.

TABLE V.
pH of Various Inhibiting Narcotic Solutions.

Substance	Inhibiting concentration	pH
Ringer's solution	—	8.6
Ethylurethane	1:66	8.6
Phenylethylurethane	1:2000	8.7
Veronal	1:400	7.3
Sodium Veronal	1:80	9.3
Amytal	1:4000	8.0
Sodium Amytal	1:2000	8.9
Luminal	1:1000	8.2
Sodium Luminal	1:80	9.3
Dial	1:500	7.0
Sulfonal	1:200	8.7
Trional	1:400	8.6
Chloretone	1:1000	8.4
Paraldehyde	1:100	7.5

Summary. (1) Narcotics belonging to various chemical groups inhibit the histamine contraction of plain musculature (intestinal strips of guinea pigs). (2) This inhibiting action of the narcotics can be enhanced by (a) substitution of aliphatic radicals with increasing C-atoms; (b) substitution of a phenyl group; and (c) halogenation. (3) The described inhibitory action is probably due to the relaxing effect of narcotics on plain musculature.

10146 P

Analysis of Experimental Production of Bifid Tails by Ultraviolet Irradiation.

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The embryological basis of spina bifida and the associated double-tailed condition has received a number of explanations. Hertwig[1] advanced the basic theory that the bifid condition results from inhibition of gastrulation which retards closure of the blastopore and thereby prevents fusion of the anlagen of the axial organs located on either side of the blastopore. Bergel[2] followed Hertwig, attributing bifid tails to a persisting blastopore which mechanically blocks the tail anlagen. Baldwin,[3] studying the effects of ultraviolet irradiation upon frogs' eggs, concluded that the essential factor was injury to the highly susceptible yolk material (endoderm), which serves as a barrier to the approximation of the blastoporal lips. Bellamy[4] suggested inhibition of the *dorsal* blastoporal lip, which is a region of high susceptibility to a variety of noxious conditions.

This study is a preliminary report of attempts to determine whether injury to one or more specific regions of the egg is responsible for the bifid condition of the lower back and tail, or whether it is produced by general injury to the egg. Localized sublethal injuries were produced easily by means of ultraviolet irradiation.

A cold quartz burner* was used whose spectrograph shows a high proportion of rays at 2537 A. U. The intensity was such that after irradiating early gastrulae for 13 minutes there was no trace of a neural plate within 24 hours. Most of the egg-jelly was removed with scalpels. The eggs were placed in a chamber the bottom of which was a quartz plate 1.65 mm thick. Irradiation was from below, with the ultraviolet burner 2.5 mm from the lower surface of the quartz plate. Shields cut from tinfoil were applied closely to the surface of the egg to protect all except the irradiated region. Eggs irradiated in the proper regions *(vide infra)* consistently developed 2 tail buds which were on opposite sides of the egg or in contact with one another in the dorsal midline. The embryos and their bifid tails developed well; some were raised to the early tadpole stage.

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[1] Hertwig, O., *Arch. f. mikr. Anat.*, 1892, **39**, 353.

[2] Bergel, A., *Arch. f. Entw.-mech.*, 1927, **109**, 253.

[3] Baldwin, W. M., *Anat. Rec.*, 1915, **9**, 365.

[4] Bellamy, A. W., *Biol. Bull.*, 1919, **37**, 312.

* We are indebted to Dr. T. D. Beckwith of the Department of Bacteriology for use of the ultraviolet apparatus.

The results of irradiating early gastrulae of *Hyla regilla,* the tree frog, are shown in Table I.

TABLE I.

Irradiated region of egg	No. of eggs	No. developing bifid tails
Entire lower surface (mesoderm and endoderm)	19	19
Endoderm only	3	0
Mesoderm ”	4	4
Dorsal lip ”	7	0
Ventral lip ”	2	0
Both lateral lips only	3	3
Ventral lip and both lateral lips	2	2
Dorsal lip and both lateral lips	2	2
Dorsal and ventral lips	3	0

Note that irradiation of the endoderm (yolk), or of the dorsal or ventral blastoporal lips alone or together does *not* produce the bifid condition, although the resulting tail is shorter than that of controls. The movements of gastrulation were delayed in all irradiated eggs, irrespective of the region irradiated. A persisting blastopore with protruding yolk plug was common after irradiation of endoderm alone, of dorsal lip or ventral lip each alone or together; but this did *not* prevent the formation of a single tail.

The significant point in which all these experiments agree is that *only by irradiation of the lateral blastoporal lips, whether alone or together with other regions of the egg, could the bifid condition be induced.* The lateral blastoporal lips of the early gastrula of *Hyla regilla* contain essentially all of the tail anlagen.[5] The bifid condition of the tail is therefore a direct effect of the ultraviolet irradiation upon the tail-forming materials of the egg. In these experiments the bifid condition was *not* induced by a general inhibition of gastrulation movements, by persistence of the blastopore, nor by inhibition of the dorsal or ventral blastoporal lips.

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[5] Schechtman, A. M., to be published.

10147

Effect of Choline Chloride on Estrous Cycle of Mice.

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Duncan, Gallagher, and Koch[1] stated that choline (a part of the lecithin molecule) has an inhibiting action on the estrous cycle of rats. At the R. B. Jackson Laboratory a similar experiment was tried on mice, but there was no apparent effect on the estrous cycle. The following method was used:

Ten dilute brown virgin female mice (dba stock) about 7 weeks old were injected subcutaneously every day with 2 mg of choline chloride dissolved in water. Injections were given as above for one month; the dose was then increased to 4 mg per day for one week and finally to 6 mg per day for another week.

Vaginal smears were examined every day in order to see if the choline chloride had any inhibiting effect on the estrous cycle. The results were plotted graphically and showed clearly that the estrous cycle had not been affected. The onset of a cycle was recorded as occurring when cornified cells appeared in the vaginal smears.

An equal number of the same stock of mice were injected with equal amounts of ammonium chloride (the inorganic analogue of choline chloride) and again the estrous cycle was not affected.

A third group of 8 mice received no injections at all. Their estrous cycles were plotted graphically and showed no significant difference from the cycles of the mice which were treated with choline chloride and with ammonium chloride.

The average length of the estrous cycles was about 5 to 6 days. The comparison of the mean intervals between cycles in the 3 groups is shown in Table I.

TABLE I.

	Difference	Probable error of difference
Choline chloride vs. controls	.241	±.312
,, ,, vs. ammonium chloride	.102	±.285
Ammonium chloride vs. controls	.139	±.314

Preliminary experiments showed that the lethal dose of choline chloride was about 8 to 10 mg, and that some temporary ill effects occurred when the mice were given only 6 mg. The regular dose was, therefore, fixed at 2 mg per day as was mentioned previously. When the dose was increased to 6 mg per day during the latter part of the experiment, the mice showed no ill effects, probably because they had built up a tolerance to choline chloride while receiving the smaller doses.

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[1] Duncan, Gallagher, and Koch, *Science*, 1937, **85**, 23.

Summary. A statistical investigation of the estrous cycle graphs showed that there was no significant difference between any of the 3 groups of test animals and that, under the conditions of this experiment, choline chloride did not inhibit the estrous cycle.

10148

Cultivation of Rabies Virus in the Allantois of the Developing Chick Embryo.

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We have attempted to cultivate rabies virus in the allantois of the developing chick embryo following the technic described by Burnet.[1] We have not succeeded in obtaining serial passage beyond the third generation. However, a number of observations have been made on the survival and localization of the virus, which are of some interest, and are briefly summarized in this note.

In our attempt to grow the virus, the allantois of embryos of various ages were infected with brain virus and the eggs left in the incubator for varying lengths of time. At intervals the infected eggs were opened, the region of the allantois around the point of inoculation removed, triturated, passed to other eggs and at the same time the presence and concentration of virus determined by injecting various dilutions intracerebrally into mice.

At the beginning only the allantois was examined and tested for virus. When the egg was not contaminated with bacteria, the allantois appeared normal and the embryo was alive and of normal development. At times there were traumatic lesions on the allantois but these were not specific.

The results may best be grouped in series. In one group embryos 11 to 14 days old were infected with 0.1 cc of 1:50 dilution of brain virus and the allantois examined 3 to 5 days later by inoculating a a 1:10 suspension in saline intracerebrally into mice. Of 20 eggs in good condition thus tested 10 contained virus and 10 were negative.

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[1] Burnet, F. M., *Special Report Medical Research Council*, 1936, No. 220.

In a second series the allantois were examined 2 hours to 14 days after infection and the concentration of virus determined by titration in mice. In one experiment the virus was present after 2 hours in a dilution of 1:1000 and after 5 days in the same concentration. In another experiment the virus was found in a dilution of 1:10 after 3 hours, 1:100 after 3 days, and 1:1000 after 7 days; in this experiment passages to other eggs were negative after 5 and 8 days and positive in one egg after 10 days in a dilution of 1:10. Further passage failed. In a third experiment, 10-day-old embryos were infected; those opened 2 days later were negative and those after 10 days were positive in a dilution of 1:10,000. Only once has it been possible to carry serial passages from allantois to allantois through 3 generations. The embryos were 7 days old when infected. Nine days after the infection the allantois was positive in a dilution of 1:100 and a passage was made. One egg opened after 10 days was negative; 2 opened after 14 days were positive in dilutions of 1:100 and 1:1000 respectively. A third passage was made and this time only one allantois was positive in a dilution of 1:10. The allantois of the fourth passage were negative.

A summary of the work showed the erratic character of the results. Of 22 embryos tested 10 were positive and 12 negative. It was apparent that the rabies virus persisted in the allantois of the chick embryo for varying lengths of time and at times it was present in greater concentration than at the time of infection. The results, however, were irregular and it is not possible to conclude whether or not actual multiplication took place.

Seeking an answer to this question, another series of cultures was set up in which both the brain of the embryo and the allantois were tested for virus. The embryos were 6 to 7 days old when infected and 20 to 21 days old when tested. In this series the allantois was positive only once in 8 experiments in a dilution of 1:10, whereas the brain was positive in each case in dilutions varying from 1:10 to 1:1000. In the experiment in which the allantois was positive in a dilution of 1:10, the brain was positive in a dilution of 1:1000.

In several instances chicks from infected eggs hatched. One chick was killed when one day old and the virus titer of the brain was 1:100; another chick was killed 3 days after it hatched and the brain contained virus in a dilution of at least 1:1000. A third chick was kept 15 days. During the first week it showed symptoms of excitability and ataxia, which slowly subsided. On the fifteenth day the chick was killed and the brain tested for virus; it was negative in a dilution of 1:10—presumably the virus had disappeared.

It appears, therefore, that rabies virus does not infect the allantois with regularity but regularly finds its way into the brain of the chick embryo. The development of the embryo is apparently not affected by the presence of the virus and may proceed in the same manner as the uninfected controls. Whether the virus can be adapted to the chick and its virulence for it enhanced by passage is a subject for further investigation.

10149 P

Activation of Renin by Blood Colloids.

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The purpose of these experiments was to learn something of the nature of the pressor action of renin. Renin was prepared from pig's kidneys in such concentration that an amount containing less than 2 mg of nitrogen produced a sharp, sustained rise (30 mm Hg. or more) of blood pressure in an unanesthetized dog. Its vasoconstrictor action was studied on perfused dogs' tails (40 experiments), so eliminating all factors causing vasoconstriction other than peripheral ones.

It was found that undialyzed renin (0.2 cc) caused moderate vasoconstriction, contrariwise dialyzed renin (1 cc) was inactive. Normal heparinized dog's whole blood (dilution of 1 to 3 parts Ringer's solution) was inactive but if dialyzed renin (1 cc) were mixed with dog's blood (1 cc 1:3 dilution) marked vasoconstriction occurred. The undialyzed renin could be potentiated by addition of dog's blood.

Heparinized plasma in doses of 0.5 cc was vaso-inactive but strongly activated dialyzed renin just as whole blood had done. But protein-free ultrafiltrate of plasma no longer exhibited activating capacity while the colloid residue on resuspension was active.

The vasoconstrictor action of undialyzed renin appears to be due in part to non-protein materials contained in it, for the protein-free fluid obtained by boiling the renin produced vasoconstriction, but it was not potentiated by blood as was renin. Dialysis removes these substances. Boiled dialyzed renin with added plasma or protein-free filtrate of boiled plasma was vaso-inactive.

These results suggest that renin is an enzyme-like substance which is activated by a kinase-like material contained in the protein fraction of plasma and whole blood.

10150 P

Iodine in Samples Containing Little Organic Matter Other Than Urea.*

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In the combustion of samples for iodine analysis, if very little organic matter is present this may be broken up by alkaline fusion and the decomposition products burned by a micro-Kjeldahl technic in the micro still (Fig. 4[1]).

Two hundred cc of water or 5 cc of urine plus 1 pellet NaOH is evaporated to dryness in a nickel crucible and 2 mg rare earth oxide added. Dry samples, such as seaweed or thyroid gland, are chosen small enough to contain about 0.2 γ of iodine and are fused with NaOH in a nickel crucible. A urine sample may require 1 g NaOH for fusion and a water residue about the same. Heating is continued until bubbles of NH_3 cease to be given off. Water is added to dissolve the fusion and 5 mg sodium azide added to destroy nitrite and reduce iodate. The samples are then transferred to the micro still and analyzed by the McClendon-Bratton method.[1]

TABLE I.

	Iodine in sample	I_2 added	Total	Difference	% error
200 cc Minneapolis tap water	0.43 γ	0.54	0.98 γ	0.55 γ	2
200 cc LaGrange deep well water	0.59	0.55	1.13	0.54	2
2.5 cc urine	0.975	—	—	—	—
5 cc urine	1.95	0.5	2.44	0.49	2

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* Aided by grants from the University of Minnesota and the Therapeutic Research Committee, American Medical Association.

[1] McClendon, J. F., and Bratton, A. C., *J. Biol. Chem.*, 1938, **123**, 699.

10151

Effect of Light on Uptake of Water by Frogs Injected with Posterior Pituitary Extract.

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Evidence pro and con has been offered that light may affect the amount of hormones produced by the anterior, middle and posterior lobes of the hypophysis. The extensive and involved literature on this subject has been recently reviewed by Bissonnette.[1] In addition, Boyd and Brown[2] have shown that when an extract of the posterior hypophysis is injected into the dorsal lymph sac of frogs kept in water, the animals take up from 15 to 20% of their weight of water in 3 to 4 hours and the uptake of water is greater when the experiment is performed in the dark room. The present investigation was an extension of these latter experiments.

It is necessary to review briefly the technic of Boyd and Brown.[2] Leopard frogs *(Rana pipiens)* were placed in 400 cc beakers containing 75 cc of water and covered with weighted screen tops. One group of frogs was used as controls and the other injected with extract of the posterior pituitary gland and all the animals were dried and reweighed every hour or at shorter or longer intervals as indicated by the changes recorded. To find the effect of light, Boyd and Brown assembled one group of animals in the dark room and another group in daylight in an open laboratory. From the results so obtained it was possible to show that there was statistically a significantly lower uptake of water in daylight than in the dark and the conclusion was made that light depressed the reaction.

The original purpose of the present experiments was to study the effect of light of varying intensities and varying wave lengths. The frogs were assembled as before in a dark room, which was a small, windowless and unventilated room, and were subjected to increasing intensities of light emanating from electric lamps increasing in intensity from 15 to 250 watts on successive days and stationed at measured lengths from the beakers containing the frogs. To assure a uniform temperature, the beakers were placed in a water bath and throughout the experiment the temperature of the bath was maintained at 21°C. In each experiment, 18 frogs were injected with 0.5

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[1] Bissonnette, T. H., The Pituitary Gland, Williams and Wilkins, Baltimore, 1938, Chap. 19.

[2] Boyd, E. M., and Brown, G. M., *Am. J. Physiol.*, 1938, **122**, 191.

international units of Pituitrin Surgical (generously supplied by Dr. E. A. Sharp of Parke, Davis and Company) per 10 g body weight and 6 frogs were used as uninjected controls. This dose of extract had been found by Boyd and Brown[2] to be more than sufficient to produce the maximum increase in body weight from water uptake.

After performing numerous such experiments with frequent repetitions of several intensities of light, it became obvious finally that the intensity of light had no significant correlation with the percentage uptake of water. The curves obtained were all similar to those of Boyd and Brown[2] at the same temperature except that they were about 6% higher at the peak, which is what might be expected at this temperature in experiments performed in the dark.

Fifteen experiments, each consisting of 20 injected frogs, were then performed in daylight in an open laboratory and the maximum percentage uptake of water was 5 to 10 points lower than in experiments in the dark room. From a statistical analysis of the resulting data, it was again apparent that the percentage uptake of water by injected frogs was significantly lower in daylight than in all of the experiments in the dark room irrespective of the intensity of light. Further experiments similarly demonstrated that there was no significant difference in the maximal uptake of water in experiments performed in complete darkness in the dark room as compared with those in the dark room with electric light of equal intensity to daylight in the open laboratory.

It then became obvious that the significant factor in the results of Boyd and Brown[2] and in the present experiments was the dark room and not darkness or intensity of light. Why the results should be greater in one room (the dark room) than in another room (the lighted and open laboratory) was then investigated. To be brief, the explanation was finally demonstrated as being the relatively greater ventilation in the open laboratory. When the beakers containing the frogs were covered with glass tops instead of screen tops, the maximum percentage uptake of water was always 5 to 10 points higher in the open laboratory than when the experiment was performed with screen tops but the type of cover of the beakers made no difference in the dark room.

These results were interpreted as indicating that the extra circulation of air in the open laboratory through the screen tops of the beakers had caused sufficient evaporation of absorbed water from the partially exposed surface of the frogs to prevent them from showing as great an uptake of water as in glass-covered beakers or in the unventilated dark room. While our results have confirmed the observations of Boyd and Brown,[2] they have further demonstrated the fallacy in the interpretation of these authors that light depresses the uptake of water in frogs injected with an extract of the posterior hypophysis.

Conclusion. The greater uptake of water by frogs injected with extract of the posterior hypophysis in the dark room observed by Boyd and Brown[2] has been shown due to the decreased circulation of air in the unventilated dark room rather than to darkness. There is no evidence to date that light has any effect upon this property of posterior pituitary extracts.

10152

Gonadotropic Action of Testosterone Propionate on the Immature Mouse Ovary.

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Vaginal introitus and estrus have been produced in the normal infantile rat with testosterone.[1, 2, 3] Little consideration has been given to the effect of this hormone on the ovary. Hohlweg[4] reported that luteinization without estrus resulted with large dosages in the rat. Recently, Salmon[5] demonstrated a gonadotropic effect with this hormone on the immature rat ovary by means of a single injection of 1 to 5 mg. Follicle stimulation resulted as the initial effect in 60 to 72 hours and corpora lutea were found after 96 hours. It was, therefore, of interest to investigate a possible gonadotropic effect of single injections of testosterone propionate on the immature mouse.

In these experiments, 148 albino mice of the "Hygenic Strain"[6] were used, of which 29 were controls. Litter-mate controls were used in all instances. Mice between 18 and 21 days of age were injected subcutaneously with dosages of 0.5, 1.0, 1.5, and 2.0 mg of

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[1] Butenandt, A., and Kudszus, H., *Z. physiol. chem.*, 1935, **237**, 75.

[2] Tschopp, E., *Arch. int. Pharm. et de Therap.*, 1936, **52**, 381.

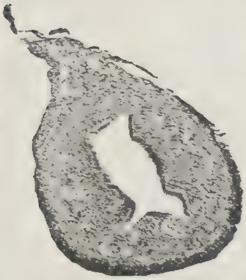
[3] Deanesly, R., and Parkes, A. S., *Brit. Med. J.*, 1936, **1**, 257.

[4] Hohlweg, W., *Klin. Wchnschr.*, 1937, **16**, 586.

[5] Salmon, U. J., Proc. Soc. Exp. Biol. and Med., 1938, **38**, 352.

[6] Starkey, W. F., and Schmidt, E. C. H., *Endocrinology*, 1938, in press.

testosterone propionate dissolved in sesame oil.* In some cases on the larger dosages, the hormone was injected at 2 different sites to avoid loss. The mice were sacrificed at 48, 72, 96, and 120 hours after the initial injection. The ovaries, uterus and vagina were re-



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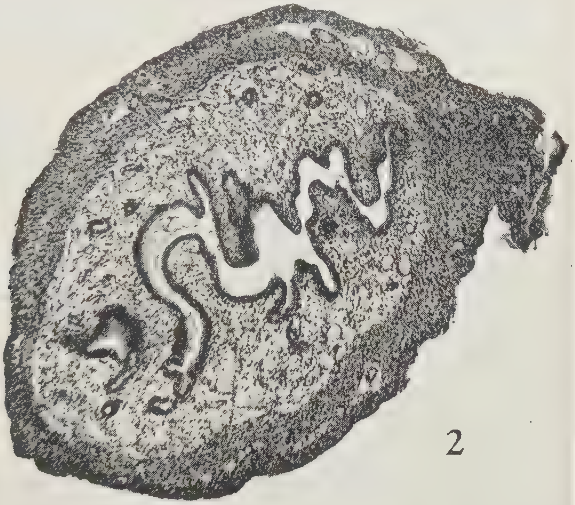

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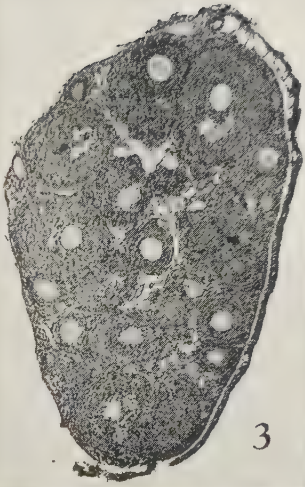

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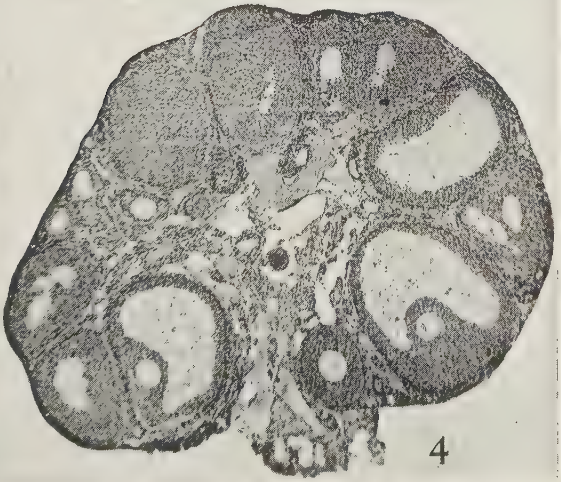

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FIG. 1. Normal immature mouse uterus. × 50.

FIG. 2. Uterus of immature mouse 96 hours after single injection of 2.0 mg of testosterone propionate. × 50.

FIG. 3. Normal immature mouse ovary. × 50.

FIG. 4. Ovary of the mouse as described under Fig. 2. × 50.

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* Supplied through the courtesy of Dr. Max Gilbert, Schering Corporation.

moved and examined histologically. Groups of 7 to 17 animals were employed at each time interval and dosage.

Although Salmon found the vagina to be open in all of his rats at 72 hours after injection, the vagina remained closed in virtually every case in our mice regardless of dosage or time interval. It cannot be stated that the few cases of vaginal opening observed were due to hormonal effect as the possibility of rupture, due to handling, cannot be excluded.

Hypertrophy of the uterus was observed at 48 hours and reached a maximal state at 72 hours. The myometrium was definitely thickened and the endometrium was "stringy" in appearance. A regression from this condition was noted after 72 hours. Mucification was observed in the uterus beginning at 72 hours and in the vagina at 96 hours.

Salmon observed follicular stimulation in 60 to 72 hours with the rat. In these experiments, follicular stimulation was evident at 48 hours with 72 hours being the time of maximum response. The ovarian stimulation was observed either as a general increase in size and number of follicles or as a marked enlargement of a few follicles. At 96 and 120 hours, good sized follicles were present in the majority of cases. Although the presence of corpora lutea in the rat ovary after 96 hours was reported,[5] lutein tissue was not observed in any case in the mouse up to 120 hours. Certain observations indicate that follicles of the immature mouse do not normally respond to luteinizing hormone.[7] Our observation, therefore, does not necessarily indicate its absence provided this difference in the reactions of mice and rats as to the formation of lutein tissue does exist.

Doses of 1.0 to 2.0 mg gave practically identical response. Animals treated with 0.5 mg responded but not to as great a degree as with the larger doses. The regression of the uterine and vaginal condition toward the normal was also more rapid after the smaller dose.

Summary Single injections of testosterone propionate produced a gonadotropic effect on the mouse ovary. Follicle stimulation resulted but lutein tissue did not form. Other investigators have suggested that the ovarian response to this hormone is dependent on the pituitary.[7]

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[7] Parkes, A. S., and Zuckerman, S., *J. Physiol.*, 1938, **93**, 16P.

10153

Capacity of Skeletal Muscle of Castrated Male Rats to Sustain Work Output.

Dwight J. Ingle. (Introduced by J. L. Bollman.)

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A comparison has been made of normal and castrated male rats in respect to the capacity of the gastrocnemius muscle to perform work when made to contract by faradic stimulation.

Albino male rats were closely matched into 12 pairs on a basis of body weight and one animal of each pair was castrated on the twenty-first day following birth. The castrated and control animals were maintained under uniform conditions. Each rat was prepared for the work test on the first day that its body weight reached 180 g. The animal was anesthetized with phenobarbital sodium; its gastrocnemius muscle was weighted with 100 g and was stimulated directly to contract 3 times per second until the "fatigue" of the muscle or for a maximum of 120 hours. The amount of work performed was recorded automatically. Each rat received 5 cc of physiologic saline solution by subcutaneous injection every 12 hours. A detailed description of the apparatus and procedures used has been presented.

TABLE I.
Work Records* for 12 Pairs of Normal and Castrated Rats.

	Hours work					
Group	0-24	24-48	48-72	72-96	96-120	Total
Normals						
Avg	72.0	49.2	48.0	40.9	32.1	242.2
Range	52.0-98.0	26.1-80.6	25.9-93.0	7.0-84.1	0.0-65.6	139.7-420.2
Castrates						
Avg	76.1	50.0	51.2	48.0	31.1	256.5
Range	61.6-104.1	30.7-83.8	26.1-88.2	11.4-97.0	0.0-64.8	136.6-420.6

*Expressed as kilogram-meters of work. The actual error in recording is unknown. The error for relative values does not exceed 10%.

The data on averages and range for 12 pairs of rats are summarized in Table I. No inferiority of the castrated male rats to sustain work was observed in this experiment. This finding does not agree with the experimental results of Gans and Hoskins,[1] who used a somewhat similar method for the study of work performance in castrated rats. The times for continuation of muscular contractions and the values for total work for both normal and castrated rats were much smaller in their experiment than in the present one. Heron, Hales, and Ingle[2] have discussed some of the important differences between the methods used by Gans and Hoskins and the method described by themselves, which I have used in this experiment.

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[1] Gans, Marvin, and Hoskins, R. G., *Endocrinology*, 1926, **10**, 56.

10154

Blood Sugar Changes in Hypophysectomized Rats During Adaptation to Various Stimuli.

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Recent experiments have shown that the blood sugar of fasting adrenalectomized animals decreases rapidly under the influence of exposure to any damaging agent capable of producing an "alarm reaction". Such a decrease, however, is not seen in animals which have been pretreated for a few days with the same dose of the same damaging agent and are consequently in the "resistant phase" of the general adaptation syndrome. It seems, therefore, that the hypoglycemia is simply part of the alarm reaction, and consequently cannot be elicited at a time when the nocuous agent has lost its "alarming" effect. After further continued treatment, however, during the "stage of exhaustion", these animals again responded with hypoglycemia which in this latter case proved to be fatal.[1]

Since hypophysectomy elicits disturbances in carbohydrate metabolism which in certain respects are very similar to those caused by adrenalectomy, it seemed of interest to perform similar experiments in the hypophysectomized animal. Female hooded rats weighing between 95-130 g were used for all experiments reported in this communication. In every case, a rest of 8 days was allowed following hypophysectomy as it was found that the blood sugar reaches a constant level at this time and stays fairly constant for as long as the animals are in good condition. In order to avoid complicating our experiments with repeated fasting and bleeding, each rat was used for one determination only. The animals were bled in groups of 6 at 1, 12, and 24 hours, and 2, 3, 5, and 6 days after initiation of treatment in each experiment. Each point on the graph represents the average of 6 individual determinations on 6 rats. In the first experiment (full line on the graph), the animals were adapted to cold by placing them in an ice-box at a temperature of +1°C for a period of one hour, 3 times a day. In the second group (dotted line), the animals were adapted to muscular exercise by forcing them to run 3 times daily for a period of 15 minutes in revolving cages having a diameter of 12 inches and running at a speed of 15 revolutions per minute. All blood sugar determinations (Shaffer, Hartmann and Somogyi method) were performed after a 24-hour fasting period. The base line has been placed at 60, since the average blood sugar of 6 control rats fasted during 24 hours on the 8th day after hypophysectomy was 60 mg %. That without treatment,

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[2] Heron, W. T., Hales, W. M., and Ingle, D. J., *Am. J. Physiol.*, 1934, **110**, 357.

[1] Selye, Hans, PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 728.

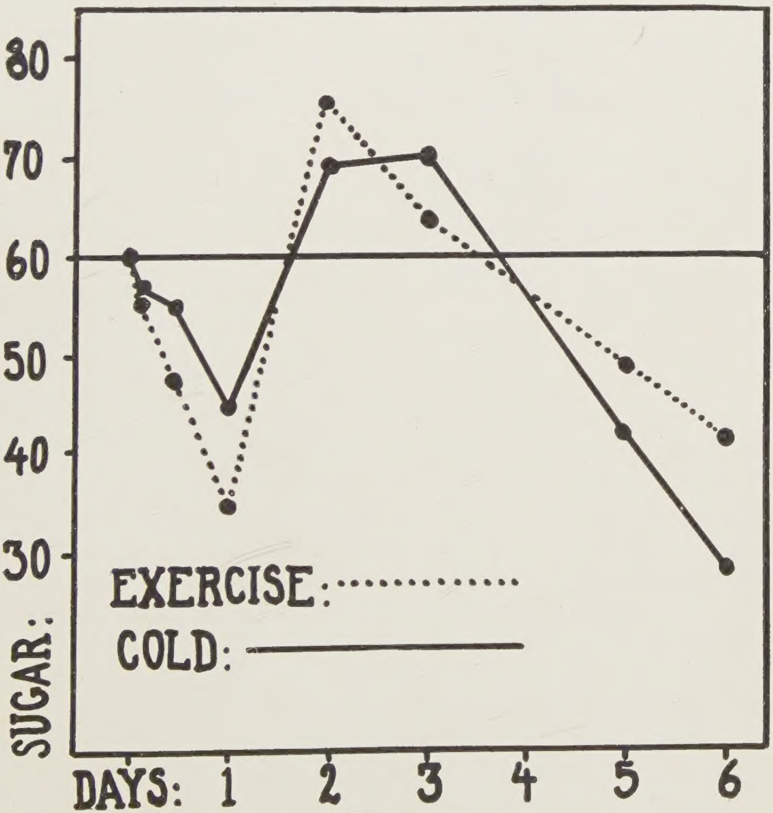

Fig. 1.

Blood sugar variations in hypophysectomized rats during adaptation to exercise and cold.

this blood sugar level would not change materially during the 6 days of our experiments was shown by the fact that a second group of 6 rats fasted during 24 hours on the 14th day following hypophysectomy had an average blood sugar of 58 mg %.

The graph shows that exposure either to cold or to muscular exercise causes an initial decrease in the blood sugar concentration, which is followed by an increase above the base line on the second and third day (that is to say, at a time when the animal acquired adaptation to these stimuli). After this, however, the blood sugar decreases again and death occurs in hypoglycemia. It appears that during the process of adaptation, the blood sugar variations in hypophysectomized rats are very similar to those of adrenalectomized animals. The fact that the adaptation acquired during the first few days is lost at a later stage gives further support to the conception that adaptive functions are not performed merely at the expense of the caloric energy supplied by food but that some special "adaptation energy" is used during the process of adaptation and that the available stores of this adaptation energy become exhausted during the process of adaptation even though unlimited amounts of food are available.

Since the blood sugar level of fed hypophysectomized animals is not significantly below normal while prolonged fasting causes particularly marked and often fatal hypoglycemia in such animals, it is held that the essential disturbance in the carbohydrate metabolism of the hypophysectomized animal is an inability to regenerate blood sugar from endogenous sources. The present experimental series is not in agreement with this interpretation, since it shows that although exposure to cold or performance of muscular exercise caused marked hypoglycemia in not pretreated fasting hypophysectomized animals, this effect is not seen after adaptation occurred; in fact, in the adapted animal, the response may be reversed so that the same stimuli elicit an increase in blood sugar. In view of these findings, it would seem more likely that hypophysectomized (like adrenalectomized) animals suffer from a disturbance in their adaptability, which makes them particularly sensitive to agents to which they are not adapted. Consequently in such animals, these agents will cause a very severe alarm reaction with the characteristic accompanying hypoglycemia. This would also explain why after a few days of pretreatment when the damaging agent lost its ability to elicit symptoms of the alarm reaction, it also ceases to cause hypoglycemia. However, the hypophysectomized, like the adrenalectomized, animal cannot maintain acquired resistance for a long time, and the final stage of exhaustion, with its characteristic fatal hypoglycemia, follows after a relatively short stage of resistance; in intact animals, the resistant stage would last for several weeks. The fact that prolonged fasting in itself may elicit severe hypoglycemia in hypophysectomized animals offers no difficulty of interpretation in terms of this theory since prolonged withdrawal of food also causes characteristic symptoms of an alarm reaction with hyperplasia of the adrenal cortex, atrophy of the lymphatic organs, decrease in blood volume, etc., even in animals not exposed to any other nocuous agent.

It should be emphasized that the general condition of our hypophysectomized rats was particularly bad during the first and the last 2 days of the experiment, while they seemed to stand treatment without visible signs of damage during the third and fourth day. In this respect, their general condition ran roughly parallel with their blood sugar. A similar parallelism existed between blood sugar and blood volume.

Unlike in our previous experiments in normal[2] and adrenalectomized rats, we were unable to make continuous blood chloride curves in this experimental series, since the blood volume was so low in many cases that we could not obtain a sufficiently large amount for an accurate determination. Yet individual chloride determinations show that in hypophysectomized, though not in adrenalectomized rats, the blood chlorides (Van Slyke method) are often very high during the terminal stage of hypoglycemia. In one such case, the chlorides were as high as 505 mg per 100 cc of whole blood although the blood sugar fell to 28 mg per 100 cc.

Summary. Experiments on hypophysectomized rats show that exposure to nocuous agents (cold, excessive muscular exercise) induces marked hypoglycemia in the animal before adaptation, but following a few days of pretreatment during the resistant stage, these same agents elicit an inverse reaction and actually increase the blood sugar above the normal level. In the absence of the hypophysis, this acquired adaptation cannot be long maintained, however, and under the influence of continued treatment the animals soon enter into the stage of exhaustion, during which fatal hypoglycemia ensues. In this respect, the response of the hypophysectomized is very similar to that of the adrenalectomized animal. These experiments are not in accord with the assumption that the characteristic disturbance in the carbohydrate metabolism of the hypophysectomized animal is an inability to regenerate blood sugar from endogenous sources, since following adaptation the fasting hypophysectomized animal learns to maintain its blood sugar at the normal, or even above the normal level, although it is exposed to such stimuli (cold, muscular exercise) as cause severe hypoglycemia in the unadapted organism.

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[2] Selye, Hans, *Arch. Internat. de Pharm. et de Ther.*, 1938, in press.